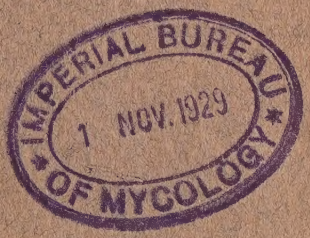
COLLEGE OF AGRICULTURE UNIVERSITY OF NEBRASKA
AGRICULTURAL EXPERIMENT STATION
RESEARCH BULLETIN 27

Potato Wilt and Stem-End Rot Caused by Fusarium Eumartii

R. W. GOSS
DEPARTMENT OF PLANT PATHOLOGY



LINCOLN, NEBRASKA
JUNE, 1924

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SUMMARY

1. The cause of potato wilt and stem-end rot in Nebraska was found to be *Fusarium eumartii,* an organism not previously reported from Nebraska.

2. The disease was found widely distributed in the commercial potato sections of the state, and has evidently been present for many years.

3. The disease was found to be one of the most serious occurring in the older potato sections in western Nebraska.

4. The symptoms and distinguishing signs of the disease were determined and are presented on page 12.

5. The disease results in decreased germination of infected seed tubers, wilt and barrenness of infected plants, decreased yields both total and marketable, and a high percentage of stem-end rot tubers. This tuber infection may increase and do great damage in storage. Such tubers are also easily invaded by secondary parasites which enter thru the infected tissue at the stem end.

6. Isolation studies showed that the organism could usually be recovered from the roots of infected plants. It could also be isolated from the lower stem unless the disease was far advanced and secondary invaders were present. Discolored tissue in the upper stem was always sterile. The organism could be recovered from stolons and infected tubers still attached to the plant except from the most advanced portions of the discolored tissue which were usually sterile. Infected tubers in storage usually contain a large number of rapidly growing secondary parasites which overgrow the causal organism which is a slow grower, and make its isolation difficult. Isolations to be dependable should be made from material fresh from the field and preferably from the roots, rather than from stored tubers.

7. The examination of both free hand and paraffin sections of infected plants showed that the organism does not invade the xylem vessels to any great extent and that wilt is not due to a blocking of these vessels by the growth of the organism. In the roots the cortex was chiefly infected while the organism was seldom found in the vascular cylinder. The organism reaching the stem from the root chiefly invades the cortex and may eventually penetrate all the stem tissues. A necrosis of certain cells accompanied by the presence of a dark staining granular substance both inter and intracellular, and a thickening and eventual disintegration of the cell walls often precedes the advance of the organism. A necrosis of various cells was observed in the upper stems and sometimes in the leaves when the presence of the organism could not be determined except at the base of the roots. This necrosis was chiefly evident in the younger tissue of the vascular bundles, both in the phloem and adjacent cells and sometimes in the xylem cells. Necrosis often occurred in cells scattered thru the pith, but chiefly in the region of the nodes. Necrosis of the cells in the stolon was observed to be similar to that in the stems. The organism was chiefly found in the cortex of the stolons. In the tuber, necrosis occurred chiefly in the vascular bundles especially in the early stages of the disease, and this necrosis always preceded the presence of the organism.

8. In liquid cultures it was determined that the organism produces a thermolabile toxic substance capable of causing the wilt of potato tops placed in the filtrate, in from 30 minutes to 2 hours. It is believed that this substance, in addition to other toxic substances probably produced by the decay of the host tissue, may be partially responsible for the wilting of infected plants.

9. It was also found that a thermolabile toxic substance was produced in liquid media, which was capable of causing maceration of potato tuber tissue in 2 to 5 hours. This substance is probably responsible for the discoloration and decay of the host tissue in advance of the organism.

10. Growth-temperature studies of the organism in Petri dish cultures were made. The optimum temperature for growth was 25°, the maximum 33°, and the minimum 5° C. The organism grows very slowly even at the optimum temperature.

11. The organism was capable of growing in media having a range of H-ion concentration from pH 4.5 to pH 8.5. The reaction of the medium had no appreciable effect on growth at the optimum temperature. Growth was slightly retarded at the maximum and minimum temperatures with a very acid or very alkaline medium.

12. Pathogenicity studies showed the organism to be a very virulent parasite capable of producing a rapid wilt of the plant and a stem-end rot of the tuber. One hundred per cent infection was usually obtained by both soil and seed inoculations except at extremely unfavorable temperatures.

13. The optimum soil temperature for the disease under control conditions with both soil and seed inoculation was 20° to 25° C. Infection could not be determined at temperatures above 30°. Slight infection occurred at 12°.

14. Soil moisture had very little effect on infection, as 100 per cent infection occurred with all moisture contents used with both soil and seed inoculation. After infection the symptoms appeared first and the resulting number of infected tubers was greatest with high moisture contents. After the first appearance of the disease the symptoms of wilt progressed most rapidly at the low moisture contents. Abundant soil moisture prevented severe wilt even when practically all the roots were infected.

15. Seed infection resulted in a slow progress of the disease up the stem and sometimes out into the roots. Soil infection resulted in a rapid progress of the disease thru the roots and after reaching the stem the discoloration of the vascular system often progressed downward into the healthy seed piece. Infection of 2 or 3 roots was enough to cause this discoloration of the seed piece which might easily be mistaken in the field for seed piece infection.

16. Field experiments showed that the amount of disease resulting from inoculated seed could be correlated with the environmental conditions as previously determined in the greenhouse experiments.

17. The disease was found to be transmitted by stem-end rotted and internally discolored seed. The type of discoloration in the seed could not be correlated with the organisms isolated nor with the percentage of resulting disease.

18. Infested soil was found to be one of the chief sources of inoculum. Healthy seed on infested soil yielded about the same as infected seed on non-infested soil.

19. Tuber inoculations at different temperatures and relative humidities showed that storage rot could be held in check at temperatures below 10° C. Temperatures above this point increased the extent of tuber rot. Temperature was found to be a greater factor than the relative humidity in relation to storage rot.

20. The organism is not capable of penetrating the sound epidermis of a mature tuber.

21. The disease can be controlled partially by the selection of healthy seed and the use of long rotations on infested soil, or by the use of new potato soil. The amount of stem-end rot in the tubers can be reduced by digging infected fields before tuber infection has occurred. The storage rot can be held in check by temperatures below 10° C. with a relative humidity as low as can be maintained without causing serious shrinking.

22. A comparison of *Fusarium eumartii* and *F. oxysporum* and the diseases they produce is presented on page 23.

Potato Wilt and Stem-End Rot Caused by Fusarium Eumartii

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INTRODUCTION

The causal relation of *Fusarium oxysporum* Schlect. to a wilt and tuber rot of potatoes has previously been investigated by the author and the results have been published (6). It was found impossible to produce the typical disease with this organism consistently. A critical review of the literature showed the same lack of experimental proof in the work of other investigators. The author therefore concluded that *F. oxysporum* was not a very virulent or destructive organism but rather a soil saprophyte capable of becoming weakly parasitic under conditions unfavorable for the host plant. The reports of losses caused by this organism were considered to be greatly exaggerated owing to confusion with other troubles.

When the author came to Nebraska in 1920 he was greatly surprised to find that in certain of the older potato growing sections of the state, potato wilt and stem-end rot, presumably caused by *Fusarium oxysporum*, was undoubtedly the most serious disease present. It was not uncommon to find fields with 50 per cent of the plants infected. Certain symptoms of the disease, notably the earliest symptoms on the foliage and the characteristic stem-end rot of the tubers, along with other detailed symptoms to be pointed out later, did not agree with the symptoms of wilt caused by *F. oxysporum* as the author had observed it elsewhere both in the field and as a result of experimental infection studies.

Isolations from wilted plants and stem-end rot tubers were made in large numbers. Many cultures from infected tubers were sterile. *Fusarium oxysporum* along with other *Fusarium* species, however, were often obtained. Infection experiments with these organisms always failed to produce the typical disease.

This work was continued, however, and in February, 1921, isolations from a stem-end rot tuber yielded a species of Fusarium different from any previously isolated. Infection experiments proved it to be a very virulent parasite capable of producing the characteristic wilt of potato plants and later tests resulted in the typical stem-end rot tubers produced by

infected plants. By means of cultural studies the organism was identified as *Fusarium eumartii* Carp. This species had not been previously reported from Nebraska.

A review of the literature showed very little experimental work with this organism. The description of the disease, however, indicated a disease similar to that found in Nebraska. The present study was therefore undertaken to obtain more information regarding the pathogenicity of *Fusarium eumartii* and to compare its action with *F. oxysporum*, in order to distinguish between and to determine the relative importance of the diseases produced by these two organisms. An effort was also made to explain the many negative results of isolation studies reported by various investigators, as well as the inconsistent results often reported in studies of the transmission of wilt by infected tubers. Investigations dealing with the effect of certain environmental conditions on the occurrence of the disease were also undertaken. All of the experiments recorded in this publication were conducted with the Bliss Triumph variety.

It is hoped that the results presented here will materially assist in clearing up some of the long disputed points concerning the wilt of potatoes, and open up a new line of investigation in the study of the action of these wilt-producing organisms and their control.

PART I

THE DISEASE

HISTORY

The first description of the disease in the tubers and the identification of the causal organism was published by Carpenter (1) in 1915. He refers to it as a previously undescribed field and storage rot observed for the previous 2 years in Pennsylvania. He lists it as a stem-end and wound invader also capable of infecting the lenticels. He does not attribute the wilt of plants in the field to *Fusarium eumartii* but considers this organism to be more likely a secondary invader following wilt caused by *F. oxysporum* or *Verticillium albo-atrum* Reinke and Berthold. No inoculations were reported on plants, but inoculations in tubers proved it to be capable of causing a serious storage rot.

In 1916 Haskell (7) in a preliminary paper reported a disease occurring in New York, causing both a wilt of the plant and a rot of the tuber by an organism "closely related to, if not identical with *Fusarium eumartii* Carpenter."

C. R. Orton (10), who furnished the material for the studies made by Carpenter, described the disease briefly as it occurred in Pennsylvania. He noted that the "stem-rot" may follow "wilt" but he did not state whether or not he believed them to be due to the same organism. He noted that it was a very serious storage rot of potatoes. No inoculation experiments were reported.

Haskell (8) gave the most complete description of the disease till then published and identified *Fusarium eumartii* as the organism mentioned in his previous note. He reported inoculation experiments by both stem inoculation and soil inoculation and his results showed that the organism was capable of causing both a wilt of the plant and a stem-end rot of the tuber. He also reported tuber inoculations showing the organism to be a wound parasite. His description of the disease fits very closely the symptoms occurring in Nebraska. The results of his inoculation experiments have been repeatedly duplicated on a larger scale by the author, and the disease described in this paper is apparently identical with that described by Haskell.

Edson and Shapovalov (3) reported a few inoculations made by placing cultures of *Fusarium eumartii* in contact

with the lower stems of potato plants. Serious stemrot, deep stem cankers, and lesions on the roots and stolons resulted from these inoculations. All the inoculations resulted in infection. No aboveground symptoms were reported.

DISTRIBUTION

The disease has not been reported as being very widespread in the United States. The first reports of the disease were from Pennsylvania and New York as noted above. Since 1916 it has been reported[1] from 3 counties in New York, from at least 16 counties in Pennsylvania, and from 5 counties in Ohio. The disease was first reported from Nebraska by the author (5) in 1923. The widespread occurrence of the disease in a severe form in western Nebraska in both the dry land and the irrigated sections would indicate that it has been present for some time. As far as the author is aware this is the first report of the disease west of Ohio. It is reasonable to suppose, in the light of the investigations herein presented, that the disease is also present in other western states.

ECONOMIC IMPORTANCE

Very little information is available on the amount of damage due to this organism. Carpenter (1) reports the disease as being a storage rot of considerable importance in Pennsylvania, sometimes causing losses of 50 per cent from dry rot of the tubers. This statement is corroborated by Orton (10).

In Nebraska the disease is considered second in importance only to spindle-tuber and mosaic, and is probably of greater importance than mosaic in many of the commercial fields in western Nebraska not being grown for seed purposes. The disease has been found to be more serious in the older potato growing sections of the state, but is becoming of increasing importance in the newer sections. It is not uncommon in favorable years for the disease, to find fields with 50 per cent of the plants infected and with a high percentage of stem-end rot tubers. The loss in storage is considerable and is increased greatly by the presence of secondary invaders, chiefly *Fusarium trichothecioides* Wr.

Table 1 was compiled from seed certification inspection reports for 1922 and 1923 for Nebraska.[2] Approximately

[1] Letter from R. J. Haskell, October, 1923, based on reports sent to Plant Disease Survey, U. S. Dept. of Agriculture.

[2] The author is indebted to Mr. H. O. Werner of the Department of Horticulture, University of Nebraska, for the data from which Table I was compiled.

4500 acres were inspected in 1922 and over 5500 acres in 1923. The author personally examined about 100 of the fields inspected in 1923.

TABLE 1.—*Occurrence of Fusarium wilt and stem-end rot in western Nebraska*[1]

Time of inspection[2]	Number of fields	Number of fields infected	Av. amount of infection in infected fields	No. of fields rejected for certification because of Fusarium wilt	Av. amount of infection in rejected fields
		Per cent	*Per cent*	*Per cent*	*Per cent*
			1922		
First—field	256	78.5	1.4	6.6	6.1
Second—field	204	76.4	4.6	19.1	16.3
Third—bin	157	82.8	3.8	12.7	14.4
			1923		
First—field	346	10.6	1.0	0.8	3.4
Second—field	193	61.1	1.4	5.1	7.7
Third—bin	148	35.8	3.2	6.0	23.7[3]

[1]These data were obtained from fields for which applications for certification had been made.
[2]First inspection was made from July 25 to August 20 in 1922 and about one week earlier in 1923. Second inspection early in September, and third as early as possible in the bins.
[3]Two of the 9 fields from which this percentage was obtained contained about 50 per cent infection, thus making the average abnormally high.

The amounts of infection shown in Table 1 are of course much smaller than the average infection for commercial fields, owing to the better grade of seed usually planted when the crop is grown for seed certification. It is evident that the amount of infection varies greatly from year to year in the same locality, and also that the disease is not usually visible in appreciable percentages until late in the season. The number of infected fields and the percentage of infection per field was much greater in 1922 than in 1923, due in a great measure to the more favorable temperature for the disease as will be more fully discussed later.

The disease percentages in Table 1 are based both on the amount of wilt in the field and on the percentage of infected tubers. The disease may also cause considerable damage by decreased germination and increased barrenness of plants. The extent of this type of damage is more difficult to determine by other than experimental methods.

The data in Table 1 include all wilted plants and all stem-end rot or vascular discolored tubers and no attempt is made to distinguish between the damage caused by *Fusarium eumartii* and the possible damage caused by *F. oxysporum*. As a result of a three years' study of the problem in Nebraska and previous investigations with *F. oxysporum* elsewhere, the author believes that probably 95 per cent of the wilt and stem-end rot occurring in the large commercial fields of Nebraska is due to *F. eumartii*. In fact the reported losses due to *F. oxysporum* previously made by the writer (6) probably included to a great extent the disease caused by *F. eumartii* as noted at that time.

The importance of the disease varies locally and in some sections is the most serious disease present, causing very serious losses both in the field and in storage.

DESCRIPTION

The symptoms of this disease as in other wilt diseases are found to vary under different environmental conditions. There are, however, certain well marked characteristics that serve to distinguish this disease from others with which it might easily be confused.

On the vines:—The first symptoms of the disease on the foliage are the appearance of small light green areas between the veins of the leaflets, giving them a mottled appearance; these areas gradually turn yellow. This is often accompanied by a bronzing of the upper surface of the leaf in smaller irregular spots. These first symptoms are easily detected after they are once associated with the disease. The rapidity of the development of the symptoms on the foliage is greatly influenced by the extent of infection in the roots and stems and by the rate of transpiration and the available soil moisture. If the soil moisture content is high the foliage symptoms may never advance to the wilting stage. Under such conditions with severe attacks the leaves may show a yellowing, rolling and rosetting, sometimes accompanied by the development of aerial tubers and other symptoms which follow injury to the stem. In case of a slight attack, the plants may never show foliage symptoms more severe than the faint mottling. With a medium soil moisture and a heavy infection the mottling becomes extended until the whole leaf surface is involved and the leaf turns yellow, dries and droops, often hanging to the stem by a thread. Sometimes there is also present an internal blackening of the small veinlets, the

veins, and petioles which is observable at the surface. Under dry conditions the wilt may progress rapidly and the plant be completely killed in a short time. If drought occurs after previous high moisture, the rosetted tops described above may dry out and the leaves still remain tightly rolled after the entire plant is dead.

On the roots:—When infection has taken place thru the roots, the root hairs and fine roots will often be completely destroyed, (Plate 7, A). Under moist conditions the cortex of the affected roots is easily sloughed off. Under dry conditions the affected roots are brittle and break off easily. When infection has occurred from or thru the seed piece, the disease often progresses out from the stem into the roots, in which case the above symptoms are observed at the base of the roots, while the tips and smaller root hairs may be healthy.

Stems:—If infection occurs from or thru the seed piece, the first signs of disease are at the base of the stem. The disease is not confined to the vascular system and often the entire basal portion of the stem sometimes up to the surface of the ground is completely destroyed (Plate 7, B). Under wet conditions it appears somewhat similar to black-leg except that the color is a dark brown instead of black. If drought follows previous high moisture the whole basal portion of the stem dries up and shatters.

When infection occurs thru the roots, the disease works both up and down the stem from the point of infection, that is, from the base of the infected roots. In this type the browning is most conspicuous in the vascular region, but gradually extends thru the pith cells, appearing as a light brown discoloration. The discoloration may extend down to the base of the stem and even into the healthy seed piece, producing a vascular discoloration in the seed that would lead a person who dug up such a plant to believe that infection had taken place from the seed piece (Plate 6, B).

In both the above types of infection, the disease, after once invading the basal stem, progresses upward, resulting in a uniform browning of the vascular tissue, often with a softening of the adjacent tissue and a brown flecking in the pith. This discoloration in the pith is more marked at the nodes. In a severe infection the discoloration extends into the tops and even into the veins of the leaf as already noted. This discoloration of the pith in the upper stem and the discolored leaf veins is not common in the field, and in fact severe wilt and stem-end rot may occur in their absence.

Stolons:—From the stem the disease works out thru the stolons to the tuber. If the epidermal tissue of the stolons is removed, a brown discoloration of the remaining tissue is evident, which is sometimes confined to the vascular elements, but in severe attacks the entire stolon may be completely rotted.

Tubers:—Tuber infection in the field always occurs thru the stolon. The common symptom on the tubers under western Nebraska conditions is a dark discoloration of the surface at the stem-end, with the affected stolon often remaining attached. The area showing the discoloration gradually becomes sunken, with a slight wrinkling of the tissue at the edges. As the disease progresses, a greater portion of the tuber shows the same appearance, but this rarely exceeds more than one-quarter of the tuber. Sections of such affected tubers show a variety of internal symptoms, which can be roughly grouped as follows: 1—Stem-end rot, with no vascular discoloration; in this type there is a sharp line of demarcation between healthy and infected tissue (Plate 2, A, B). 2—Stem-end rot with vascular discoloration (a) accompanied by a soft rot of the adjacent tissue forming a band of light brown tissue from 5 to 10 mm. wide which sometimes extends the entire length of the tuber; (Plate 1, B, C, D, F),—this is the most common type; (b) the discoloration in advance of the stem-end rot may appear as a black network or sometimes a brown flecking of the tissue, found most prominent in the vascular region but not entirely confined to it (Plate 1, A); (c) the vascular browning may be a very light brown discoloration entirely confined to the vascular region (Plate 1, E), similar to the type of discoloration more often produced by *Fusarium oxysporum*. The above types of vascular discoloration may all occur in the absence of any stem-end rot.

These symptoms have all been found in tubers produced on inoculated plants and appear to be characteristic of the disease as it occurs in the field. Efforts to segregate these different types of symptoms on the basis of organisms isolated in culture or on the type of disease resulting from planting such seed have been unsuccessful, as will be pointed out later. All types have occurred on plants inoculated with pure cultures of *Fusarium eumartii*. That listed above as type 2 (a) appears to be more commonly associated with the disease than the other types.

When the vascular discoloration extends into the tuber for any distance, the eyes are often infected and fail to sprout.

In severe cases such eye infection can be seen externally. The disease increases in storage, unless temperatures below 10° C. are maintained. Such increase is also favored by high relative humidity. The disease also causes serious damage in stored tubers by the action of secondary invaders such as *Fusarium trichotheciodes.*

THE CAUSAL ORGANISM

Identity and Description

In a previous report the author (5) expressed the opinion that the organism isolated in Nebraska in 1921 and which was capable of producing the wilt and stem-end rot of potatoes was *Fusarium eumartii.* Further studies have substantiated this view.

This organism has been found to be practically identical[1] with *Fusarium eumartii* as described by Carpenter (1). Certain minor differences regarding septation and size of conidia have been observed. The following brief summary of many observations of the organism in pure culture from single spore isolations is therefore presented.

Fusarium eumartii:—(Type from culture No. 64 used in the present investigation, isolated from stem-end rot potatoes produced by a wilted plant). Conidia nearly straight in lower half, slightly curved in upper half, of nearly even diameter thruout, apex rounded, basal cell ventrally depressed, slightly pedicellate. Conidia 4 to 6 septate with 5 septate conidia greatly predominating, averaging 50 to 60 x 5 to 7 u. on a 2 per cent agar medium not too rich in sugars. Seven septate conidia rare. Aerial mycelium weak to well developed. Conidial masses vary from light brown to deep blue green and dark mixed colors. Zonation occurs on oat agar, the color being light brown when young and later becoming dark green, sporodochia usually abundant. On soft agars, the plectenchymatic stroma is weakly developed or lacking and pionnotes cover the surface of the substratum. Microconidia typically present on aerial mycelium, mostly O-septate. Chlamydospores terminal and intercalated and often conidial in old cultures.

The percentages of variously septate conidia and their relative size on different media are as follows:

[1] The author is indebted to Dr. C. D. Sherbakoff of the University of Tennessee for help and advice in determining the identity of the organism.

Cultures on potato plugs 14 days old.

Aerial mycelium sparse, 1 to 2 mm. high, white, changing to green near the substratum. Sporodochia light brown in color, microconidia 1.5 per cent, macroconidia 98.5 per cent.

3 septate, 10 per cent, 30.7x2.5 u. (25–35x1.7–3 u.)
4 septate, 25 per cent, 34.0x3.7 u. (27–42x2.5–4 u.)
5 septate, 60 per cent, 38.0x3.7 u. (32–47x2.5–5 u.)
6 septate, 3.5 per cent, 45.0x4.2 u. (41–47x3.7–5 u.)

Cultures on 2 per cent nutrient agar, 15 days old.

Pseudopionnotes vinaceous blue, no aerial mycelium. Microconidia rare.

Macrocondia, 1 septate, 0.5 per cent, 15.0x2.5 u.
2 septate, 1.0 per cent, 29.7x4.3 u.
3 septate, 19.0 per cent, 33.2x4.6 u. (27–37x3.7–5 u.)
4 septate, 26.5 per cent, 34.7x5.0 u. (32–37x3.7–6 u.)
5 septate, 52.0 per cent, 38.5x5.0 u. (34–42x3.7–6 u.)
6 septate, 1.0 per cent, 42.5x5.0 u.

Cultures on 2 per cent oat agar, 70 days old.

Aerial mycelium sparse, light brown. Numerous dark green sporodochia on surface of the substratum arranged in concentric zones. Microconidia rare.

Macroconidia, 1 septate, rare.
2 septate, 1.0 per cent, 26.2x4.2 u.
3 septate, 6.0 per cent, 34.7x5.2 u. (23–46x3.5–7.0 u.)
4 septate, 12.0 per cent, 49.0x5.9 u. (41–59x5.2–7.0 u.)
5 septate, 67.0 per cent, 58.1x6.6 u. (42–63x5.3–7.7 u.)
6 septate, 14.0 per cent, 64.0x7.0 u. (56–70x5.4–7.7 u.)
7 septate, rare.

It is evident from the above data that the organism varies considerably under different cultural conditions and that in many instances it does not show as high septation or as large size conidia as described by Carpenter (1). However, the growth of the fungus on oat agar as shown by the above measurements was very similar to Carpenter's original description of the organism. The general cultural characteristics and the type of conidia were the same; therefore the organism here studied is considered to be identical with his *Fusarium eumartii.*

Physiology

ISOLATION

The causal organism can usually be isolated from the roots of infected plants by the ordinary tissue culture methods. Isolations from heavily infected basal stems often yield miscellaneous organisms, while cultures from the discolored pith which sometimes occurs in the upper stem are usually sterile. The pathogene can often be recovered from infected stolons and from tubers recently removed from such stolons. Cultures from such tubers, however, are usually sterile if the isolations are made from the extreme edges of the discolored tissue as this discoloration occurs in advance of the organism. Isolations from the black or dark brown necrotic tissues, sometimes scattered thru the tuber but usually more severe in the vascular region, are usually sterile. Cultures from infected tubers which have been in storage often yield a variety of tuber rot fungi which are usually more rapid growing organisms than *Fusarium eumartii*. Isolation studies from stored tubers are therefore not a satisfactory basis for determining the presence of the causal organism, and often result in confusion owing to the large number of sterile cultures or miscellaneous fungi usually obtained. Isolations should be made from fresh material obtained directly from the field if dependable results are desired.

GROWTH-TEMPERATURE RELATIONS

The optimum temperature for growth of *Fusarium eumartii* on a 3 per cent potato dextrose agar is 25° C. Slight growth occurs at 5° after 9 days' exposure. Growth occurs at 33° but not at 35°. Exposure to extremely high or low temperatures slightly retards the growth for a few days when the cultures are afterwards changed to the optimum temperature. Intermittent temperatures with 16 hours' exposure at 35°-and 8 hours at 25° each day, allow slight growth. On a 3 per cent potato agar there is a great decrease in growth at 30° as compared with that at 25°. This decrease is not so great when 2 per cent potato dextrose agar is used as the medium.

EFFECT OF THE H-ION CONCENTRATION OF THE MEDIUM ON THE GROWTH-TEMPERATURE RELATIONS

Fusarium eumartii is capable of growing under a wide variation in the H-ion concentration of the medium. At the optimum temperature no appreciable difference is evident

with H-ion concentrations varying from pH 4.5 to pH 8.5 on 2 per cent potato dextrose agar. At the minimum and maximum temperatures for growth there is a slight decrease in the amount of growth at pH 4.5 or at pH 8.5, the maximum growth occurring at a pH of about 7.5.

Pathogenicity

Inoculation experiments of various kinds reported in Part II prove that *Fusarium eumartii* is an extremely virulent parasite capable of causing a wilt of the potato plant and a stem-end rot and internal discoloration of the tuber. Infection can be obtained by seed, soil, and stem inoculations. Such inoculations have always yielded 100 per cent infection except under extremely unfavorable conditions for the disease. The progress of the disease when produced by inoculations is often more rapid than that normally occurring in the field. The typical disease can be produced by the inoculation of 2 to 3 roots.

Life History

SOURCE OF INOCULUM

Fusarium eumartii may live saprophytically in the soil. There are indications that the organism may occur in virgin soils. How long it lives over in heavily infested soils has not been determined. The organism may also live over winter in the seed tuber. Such infected seed when planted will often yield 40 to 50 per cent of diseased plants.

METHODS OF INFECTION

Infection from the soil may occur either thru the roots or thru the seed piece. In Nebraska infection has usually been found to occur thru the roots. The organism chiefly attacks the root hairs and smaller roots, which are often completely destroyed, and it then progresses up into the stem. Such infection results in a discoloration in the stem which may extend down to the seed piece causing a vascular discoloration of the seed piece, as shown in Plate 6, B.

Infection from or thru the seed piece results in a discoloration of the lower stem which may later decay completely. Such infection often progresses out from the stem into the roots for some distance.

In heavily infested soils, infection may occur directly thru the stem, producing a light brown lesion and eventually penetrating thruout the stem.

FACTORS INFLUENCING INFECTION AND THE PROGRESS OF THE DISEASE

With constant soil temperatures the optimum temperature for infection either from the soil or from the seed piece is about 22.5° and the disease is more severe at 20° than at 25° C. (Plate 3, C). As the temperature decreases, the number of infections and the severity of the disease diminish, until slight infection occurs at 12° and 15°. Similar results occur at temperatures above the optimum, no infection occurring at temperatures above 30°. If infection occurs at the optimum temperature or below, and this is followed by a rapid increase in temperature, the plant wilts very rapidly.

The disease occurs with a wide range of soil moisture contents. The amount of soil moisture apparently does not have any great effect on the amount of infection but it does cause marked differences after infection has occurred (Plate 4). The wilting of the plant is most rapid when the moisture content is low. With high soil moistures the partially destroyed root systems are enabled to take up enough water to prevent the rapid wilting of the plant; the disease, however, continues to progress and often causes a large amount of stem-end rot in the new tubers.

Under conditions of high temperature and low moisture, the wilting of infected plants is very rapid. With low temperatures and high soil moistures the wilting symptoms may not appear and yet the disease may be causing considerable damage.

Factors affecting stem-end rot in storage:— When tubers which have become infected in the field are placed in storage the progress of the disease and the resulting tuber-rot depends greatly upon the temperature and relative humidity of the air in the storage cellar. The disease is favored by high storage temperatures and a high relative humidity (Plate 2). Temperatures below 5° C. completely check the progress of the disease and temperatures below 10° permit only a slight growth even with a high relative humidity; with increasing humidity and higher temperatures the rate of progress of the disease increases. The rotting at the stem-end which often occurs with tubers infected with *Fusarium eumartii* provides a source of entry for other more rapid growing tuber-rot fungi, which often overgrow the real causal organism. *F. eumartii* is not capable of penetrating the sound epidermis of the potato but can gain entrance only thru wounds, or thru the stolon in the field.

Toxic Secretions of the Organism

IN RELATION TO WILT

Fusarium eumartii when grown in certain liquid media secretes a thermolabile toxic substance capable of causing very rapid wilting of the cut tops of healthy potato plants when they are placed in the filtrate. It is possible that the secretion of this substance in the plant may account in part for the wilting of the plant and also for the killing of the tissue in the upper stem considerably removed from the locus of the organism.

IN RELATION TO TUBER ROT

It has been found that the organism is also capable of secreting a thermolabile toxic substance capable of breaking down the middle lamella of the cell walls in potatoes. It is probable that this action is correlated with the discoloration of the host tissue in advance of the organism as revealed by isolation studies and microscopic examinations.

Relation of the Organism to the Host Tissue

A complete study of the pathological changes in the structure of the host tissue has not been undertaken. However, numerous examinations of infected plants, both by free hand sections and by sections of imbedded material, have brought out certain points regarding the relation of the organism to the host tissue. These examinations have usually been paralleled by isolations from similar portions of the same plant.

It was found that the organism usually penetrated the outer cells of the fine roots (Plate 8, A) and was chiefly present in the cortex. It was not found to be present in the vascular cylinder except in the last stages of the disease when the root was badly decayed. When only the cortex was destroyed complete wilt did not occur, provided the soil moisture was abundant, even tho practically all the roots were infected as the distintegration of the infected cortex allowed the roots to take up and transport sufficient water apparently as readily as when the cortex was present. The cells of the cortex for a considerable distance in advance of the organism were killed, the cell walls were broken down (Plate 8, B), and very often there was an accumulation of a deep staining granular substance within these cells.

When the organism finally reached the lower stem it was at first chiefly limited to the parenchymatous cells of the

cortex, but it eventually penetrated all the tissue of the basal stem. Plate 8, C, shows a tangential section of the lower stem at the base of an infected root. The organism penetrating the stem from the infected root was abundant at this point. In this plant the organism could not be recovered in culture or its presence demonstrated by sections at a distance of 5 mm. above the infected roots. Nevertheless, the plant had wilted and an internal necrosis of various tissues had occurred even as high up as the leaves. The tissue first showing necrosis in advance of the organism in the stem has not been determined. Sections made thru the stem about 2 inches above the section shown in Plate 8, C, revealed a necrosis of the younger tissues of the vascular bundles (Plate 9, A, B, C). The presence of the organism at this point and at all points above could not be determined either by cultures or by sections. In Plate 9, A, necrosis is apparent in the external phloem and adjacent cells. The cambium between the xylem and the external phloem also appears to be affected and the amount of cambium is less extensive than occurs in a normal plant. The large heavy-walled cells of the xylem are apparently not greatly affected. In Plate 9, B, another bundle from the same section is shown in which the xylem elements with the adjoining cells and intercellular spaces show the presence of the dark staining granular substance and deep staining cell walls. The other bundle in this section (Plate 9, C) shows this necrotic condition confined largely to the external phloem. Tangential sections made about 6 inches above this point reveal a similar necrotic condition of the pith cells (Plate 9, D). The vascular bundles at this point showed no signs of disease. The necrosis of the pith occurred for a distance of 12 inches above the ground and was more severe at the nodes, where the central pithy cylinder was entirely disintegrated and stained a deep brown color. The granular substance so often found in the necrotic cells and in the intercellular spaces stained a deep green with Pianeze's stain while the surrounding healthy cells stained red. With Delafield's haematoxylin and erythrosin, these necrotic cells took a deep brown or sometimes a golden yellow stain.

Sections of affected stolons showed a necrosis of the same tissues as occurred in the stem. The penetration of the stolon by the organism, however, usually showed the mycelium to be more abundant in the cortex and it was rarely found in the vascular bundles even tho necrosis was apparent.

Sections of affected tubers showed the same appearance of necrotic tissues in advance of the organism. Necrosis was

usually most abundant in the vascular bundles and surrounding tissues. Plate 8, D, shows a transverse section of a tuber which showed a slight stem-end rot and vascular discoloration with a soft, watery appearance of the adjacent cells. The organism could not be isolated from the tuber but it was isolated from the stolon. Examination of sections of the tuber did not reveal the presence of the organism but showed the typical necrosis pictured in Plate 8, D.

It was therefore evident that the host tissue was killed for a considerable distance in advance of the organism. The results of isolation studies presented on p. 33 also substantiate this fact. It was also evident that wilting of the plant is not caused by the growth of the organism blocking the xylem tubes.

CONTROL

Inasmuch as the disease is transmitted both in the seed and thru the soil, the only practical control method is the use of healthy seed and non-infested soil. Infected seed can be eliminated by discarding all tubers not perfectly sound. It is possible that healthy tubers which have been stored with infected tubers might carry the organism on the surface. The small amount transmitted in this way would be controlled by the usual seed treatments for scab and Rhizoctonia. Long rotations should be used if land has produced a crop of potatoes seriously affected with the disease. Infected seed tubers on non-infested soil give rise to about the same amount of disease as healthy tubers on infested soil. While the organism may be present in soils not previously planted to potatoes, the amount of infection on such soils is usually much less than occurs on old potato soil. New potato land should therefore be used when possible. The use of new potato land is entirely practical in many of the new potato growing sections of the west. The disease can be controlled only by the combined use of healthy seed and new soil or the practice of long rotations.

The amount of stem-end rot in the crop of an infected field can often be reduced by digging a week or two early if the disease has not already reached the tubers. Low storage temperatures and a relative humidity as low as can be obtained without causing shrinkage of the tubers will prevent the further progress of the disease and also the secondary invaders which usually follow stem-end rot.

COMPARISON OF FUSARIUM EUMARTII AND F. OXYSPORUM AND THEIR EFFECTS ON POTATO IN NEBRASKA

THE ORGANISM

Fusarium eumartii Carp.	*Fusarium oxysporum* Schlect.

TEMPERATURE RELATIONS

Optimum 25°, maximum 33°, minimum 5° C.	Optimum 25° to 30°, maximum 37.5°, minimum 5° C.

RATE OF GROWTH IN ARTIFICIAL MEDIA

Slow growth. Diameter of colony on potato agar in 6 days, 38 mm. at optimum temperature.	Rapid growth. Diameter of colonies on potato agar in 6 days, 90 mm. at optimum temperature.

THE DISEASE

ECONOMIC IMPORTANCE

Serious, causing 85—95 per cent of the wilt and stem-end rot in Nebraska. One of the most serious diseases especially in the older potato growing sections of western Nebraska.	Slight, causing not more than 10 per cent of the wilt in Nebraska, and practically no stem-end rot. Minor disease.

DESCRIPTION

On Foliage

Light green mottling accompanied by an irregular bronzing of upper leaf surface. Leaf slowly turns yellow under dry conditions. Leaf dropping in severe attacks with leaf hanging to stem by thread. Final complete wilt. Rolling and rosetting of foliage under moist conditions.	Yellowing around margins of lower leaves, gradually involving entire top, with final complete wilt.

In Roots

Destruction of root hairs and fine roots. Brittle under dry conditions. Cortex sloughs off under wet conditions.	Similar to *Fusarium eumartii.*

In Stems	
Brown discoloration of vascular bundles, accompanied by necrosis of pith and cortex. Discoloration extends into upper stem and foliage. Pith often discolored at the nodes.	Discoloration chiefly confined to vascular bundles and not extending above the surface of the ground.
In Tubers	
Sunken areas at stem-end usually occur with wrinkling of tissues around margins. Internal discoloration of various types, chiefly vascular, accompanied by a soft, watery, light brown appearance of adjacent tissue about 5 to 10 mm. in diameter often extending thru the entire tuber.	Usually no stem-end rot. Internal discoloration chiefly confined to vascular bundles as a light brown discoloration.
ISOLATION	
Isolated easily from roots. Isolated with more difficulty from stems, especially in later stages of disease. Discoloration in upper stems sterile and also at the extreme ends of discolored tissue in stems and tuber. Isolated with difficulty from stored tubers.	Isolated easily from roots and stems. Often isolated as a secondary invader from stem-end rot tubers. Isolated with difficulty, but more easily than *F. eumartii,* from vascular discolorations in stored tubers.
PATHOGENICITY	
Actively pathogenic under a wide range of conditions. One hundred per cent infection always obtained in greenhouse experiments, except at extreme temperatures, with all methods of inoculation. Complete wilt results from inoculation of 1 to 3 young roots.	Weakly pathogenic under all conditions. Small number of infections obtained and results always variable. Pathogenic only under conditions extremely unfavorable for host plant and with severe inoculations.

PROGRESS OF THE DISEASE

With stem inoculations of young plants first symptoms appear in 1 to 3 weeks and complete wilt in 5-7 weeks. Slower progress with soil and seed inoculations, but complete wilt always occurring under optimum conditions.	All methods of inoculation give variable results. Often no wilting symptoms appear and diagnosis usually made on basis of vascular discoloration. Complete wilt seldom produced except at extremely high temperatures or low soil moisture.

RELATION OF SOIL TEMPERATURE TO THE DISEASE

Optimum soil temperature 20° to 25° C. Slight infection at 12°. No infection above 30°.	Optimum soil temperature above 30° C. Slight infection at 16° with heavy soil inoculation.

RELATION OF SOIL MOISTURE TO THE DISEASE

One hundred per cent infection at all moisture contents. Wilt appears first at high soil moistures, but after appearance wilt is accelerated by low soil moistures. Tuber infection greatest at high soil moistures.	Infection favored by high soil moisture. After infection, wilt is accelerated by low soil moisture.

RELATION OF INFECTED SEED PIECE TO THE DISEASE

About 50 per cent of stem-end rot seed reproduce the disease directly. Such seed produces plants more susceptible to infection from the soil than healthy seed.	A variable amount of infection occurs from infected seed. Usually only a small per cent of infection occurs in this way.

RELATION OF INFESTED SOIL TO THE DISEASE

Badly infested soil results in about 25 to 50 per cent disease even under unfavorable conditions for the disease. Infection from soil usually occurs thru the roots and may result in an internal discoloration of the healthy seed pieces by progressing down the stem.	Results from infested soil are variable, depending chiefly on environmental factors. Most of infection takes place thru the roots.

PRODUCTION OF TOXIC EXCRETORY SUBSTANCES

Produces a toxic substance in culture capable of wilting potato tops placed in the filtrate in 30 minutes to 2 hours. Also produces a toxic substance capable of macerating potato tuber tissue in 2 to 5 hours. The activity of this substance is destroyed by boiling for 15 minutes.

Produces a toxic substance causing wilt of potato tops placed in the filtrate in 1 to 3 days. The activity of this substance is not destroyed by boiling.

PART II

EXPERIMENTAL EVIDENCE

LABORATORY STUDIES

Source of Cultures

Single spore isolations of the first strain of *Fusarium eumartii* isolated in February, 1921, from a stem-end rot tuber from western Nebraska and listed as culture No. 64 have been used in identifying the organism and as a source of inoculum for all experiments reported in this paper unless otherwise noted. Reisolations from inoculated plants have been repeatedly obtained.

A large number of other strains of *Fusarium eumartii* have been isolated from infected plants in Nebraska and have been used for pathogenicity tests. All strains of the organism have behaved similarly. A few comparative pathogenicity tests with a culture of *F. eumartii* obtained from the United States Department of Agriculture thru the courtesy of Dr. M. Shapovalov and listed as culture No. 204 have given somewhat similar results, but Nebraska culture No. 64 produced more severe symptoms more rapidly than the Washington culture. This may have been due to the fact that the Washington culture was not in a state of "high culture." No change in the degree of virulence of culture No. 64 has been noted in 3 years.

Growth-Temperature Relations in Pure Culture

Experiment 1

Methods:—Cultures of 3 strains of *Fusarium eumartii* were grown for one week on hard potato dextrose agar[1] in Petri dishes. Disks 2 mm. in diameter were cut from these stock cultures and transferred in an inverted position to the center of a series of Petri dishes each containing 15 c. c. of a similar medium. The transfers were all made at a room temperature of 25° C. and the cultures were immediately placed at the desired temperature after inoculation.

Temperatures of 5°, 10°, 15°, 20°, 25°, 30°, 33°, and 35° C. were used. The variation in temperature in each case did not exceed 1° above or below the desired point. Temperature records were made 3 times each day and averaged as follows:

[1]200 gms. sliced potatoes, 30 gms. agar, 50 gms. dextrose in 1000 c.c. water.

5.5°, 10.0°, 14.9°, 19.7°, 24.7°, 30.0°, 32.8°, and 34.5°. After an exposure of 10 days at the above temperatures all cultures were transferred to 25° to determine the effect of the previous temperatures on the subsequent growth at 25°. Measurements of the cultures were made every 24 hours by taking two readings of the diameter of the colonies and subtracting the size of the disk used in the inoculation.

Four Petri dishes were used for each of the 3 strains of *Fusarium eumartii* at each temperature. All strains behaved alike, however, and in the results presented graphically in figure 1 all cultures are grouped together.

Results:—The optimum temperature for growth was 25° C. The minimum and maximum temperatures for growth were 5° and 33°. No growth occurred at 5° until the tenth day, and no growth was observed at 33° until the third day. In this experiment no growth occurred at 35°. No tests were made at temperatures below 5°. The organism was found to be a very slow grower, producing a colony only 66.7 mm. in diameter in 10 days at the optimum temperature. The most noticeable characteristic of the results shown in figure 1 is the small amount of growth occurring at 30° as contrasted with that occurring at a temperature 5° lower.

The same type of growth-temperature curves as those shown in figure 1 will be found if the daily increments instead of the total growth are plotted. After the first 48 hours' growth, the daily increments were practically the same for successive days at each temperature and there was no apparent shifting in the optimum temperature for growth on successive days. If the temperatures employed had occurred at smaller intervals, it is possible that a shifting of the optimum temperature might have been observed, as this has been reported to occur with other organisms. With the 5° C. intervals employed here it was not observable.

The dotted lines in figure 1 represent the total growth occurring after all cultures[1] were transferred to 25° C. It was evident that the previous exposure to unfavorable temperatures influenced the rapidity of growth when the cultures were changed to the optimum temperature. This inhibition, however, was gradually overcome so that after 4 days' growth at 25° all cultures were producing nearly equal daily increments of growth. The cultures previously grown at the low temperatures recovered more quickly than those

[1] The cultures at 33° C. dried out too rapidly to be used after 10 days' growth.

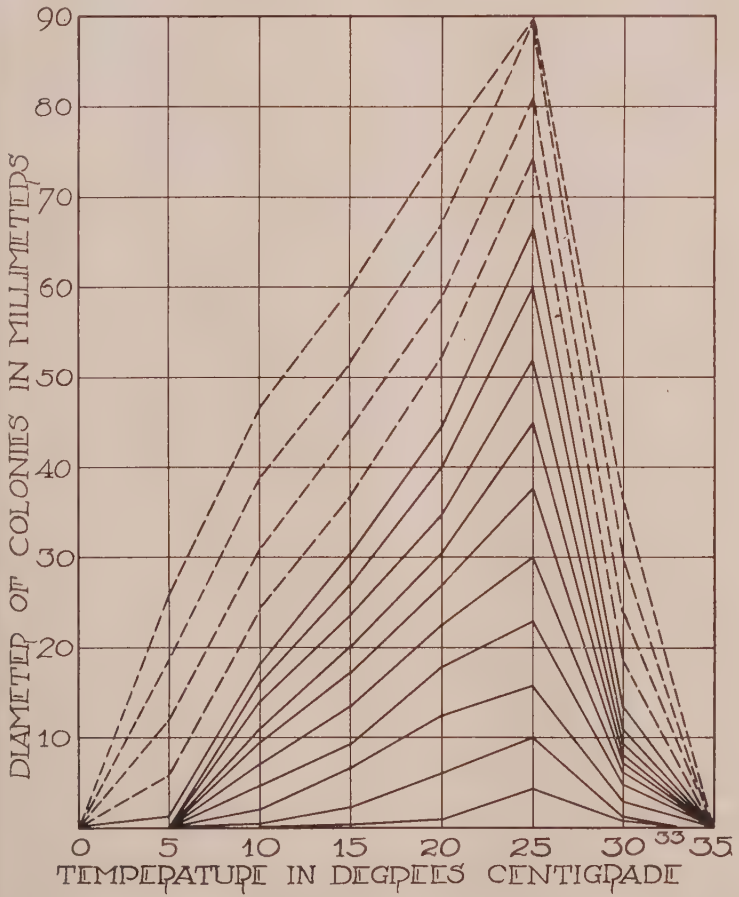


FIG. 1.—Graph showing the total growth for 10 successive days, of *Fusarium eumartii* on 3 per cent potato dextrose agar at different temperatures. The dotted lines represent the total growth for 4 successive days after all cultures were transferred to 25° C. (Experiment 1).

grown at 30°, but this may have been partially due to the more rapid drying of the medium at the higher temperature.

The results presented here are in a general way in agreement with other published reports on the growth-temperature relations of this organism. Edson and Shapovalov (4) report the same optimum temperature for growth with no germination occurring at 35° C. They did not obtain growth at 5° in 7 days' exposure. In the author's experiments, growth at 5° did not occur until the tenth day. The chief difference in the results obtained by the author and those reported by Edson and Shapovalov occurred at 30°. In their experiments the growth at 30° was nearly as great as at 25°, while in this experiment less growth occurred at 30° than at 10°. The author also obtained growth at 33°, a temperature not used in their experiments. The difference in growth at 30° may be accounted for on the basis of the media used, as Edson and Shapovalov employed a 2 per cent potato agar, while in this experiment a 3 per cent potato dextrose agar was the medium employed. Later experiments (p. 32) with 2 per cent potato dextrose agar resulted in an even greater comparative growth at 30° than that reported by Edson and Shapovalov, and also considerably more growth at 33° than was found in Experiment 1, thus showing the effect of the medium employed in temperature studies of this kind.

A further test was made of the inhibiting effect of high temperatures on the growth of the organism. Cultures were made as in the preceding experiment and placed at 35° C. Each day 3 cultures were removed to a temperature of 25° and measurements made for 10 successive days. It was found that after 10 days' exposure to 35° the cultures were dry and the organism did not grow when removed to 25°. For all exposures under 10 days, the growth was retarded for the first 48 hours after which the daily increment of growth was practically the same for all cultures. The extent of this retardation increased with the length of the exposure at 35°. The cultures held at 35° for only one day grew as rapidly at 25° as those not exposed to the high temperature. Those held for 9 days at 35° showed only about 80 per cent as great a growth after 10 days at 25°.

Another test was made to study the effect of intermittent high temperatures on the growth of the organism. Cultures were made in the same way as in previous tests and divided into 2 sets. One set of 6 cultures was placed at 35° for 16 hours each day and then changed to 25° C. for 8 hours. Another set was placed at 35° for 8 hours each day and then

changed to 25° for 16 hours. Measurements of the increase in diameter were made every 24 hours, at the time the temperatures were changed, for a period of 10 days. The average growth for all cultures in the experiment was as follows:

Temperature		Total growth in millimeters for successive days									
35 ° C	25 ° C	1	2	3	4	5	6	7	8	9	10
16 hours	8 hours	0	0	0	0	0	0.1	0.9	1.7	2.3	2.7
8 hours	16 hours	2.1	6.1	8.6	11.2	13.6	17.0	20.2	23.1	25.7	28.1

Comparing the growth-temperature relations of *Fusarium eumartii* with those of *F. oxysporum* previously reported (6), grown under the same experimental conditions, it is evident that *F. eumartii* grows more slowly and that high temperatures greatly retard its growth. Whereas the optimum temperature for both organisms was about the same, 25°, *F. oxysporum* grew as well at 30° as at 25°, and with some strains this was the optimum temperature for growth; it also made a slight growth at temperatures as high as 37.5° C. The growth of *F. eumartii* was greatly inhibited at 30° and none occurred at 35° in these experiments. A greater comparative growth at the lower temperatures occurred with *F. eumartii* than with *F. oxysporum*.

Effect of the H-Ion Concentration of the Medium on Growth-Temperature Relations

Experiment 2

In this test an effort was made to determine not only the effect of the H-ion concentration of the medium on the growth of the organism, but also whether this effect would remain constant under all temperature conditions.

Methods:—Cultures were made as in the previous experiments and the following temperatures were employed: 5°, 10°, 15°, 20°, 25°, 30° and 33° C. Records taken 3 times a day during the experiment showed the following average temperatures: 4.8°, 10°, 14.7°, 20°, 24.9°, 30° and 33°. Potato dextrose agar[1] was adjusted to the following H-ion concentrations by the colorimetric method: pH 4.5—5.0—6.0—7.1—7.5 and 8.5. The experiment was run in triplicate. Measurements of the diameter of the colonies were recorded every 24 hours. The changes in the reaction of the medium due to the growth of the organism during the experiment were not determined.

Results:—As in the previous experiment, the optimum temperature for growth was found to be 25° C. At 5° growth

[1]200 grams sliced potatoes, 10 gms. dextrose, 20 gms. agar, 1000 c.c. water.

was more rapid than in Experiment 1, being measurable in 6 days. Growth was greater at 30° and 33° than occurred in Experiment 1. The total growth for 10 days for all cultures is tabulated in Table 2 and these results are comparable with the daily total growth thruout the experiment. The great increase in growth at 30° over that occurring in Experiment 1 has already been noted on p. 30, and is probably due to the medium employed. The results here are more in accord with the data presented by Edson and Shapovalov (4) who also used 2 per cent agar.

TABLE 2.—*Effect of the H-ion concentration of the medium on the growth-temperature relations of Fusarium eumartii*

Diameter of colonies expressed in millimeters, after 10 days' growth
Experiment 2

H-ion concentration	5°C.	10°C.	15°C.	20°C.	25°C.	30°C.	33°C.
pH	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*
4.5	2.0	11.8	26.1	48.6	65.1	52.0	13.2
5.0	2.6	11.8	26.8	49.6	64.7	53.6	17.8
6.0	2.8	12.5	29.5	48.8	65.2	55.5	19.0
7.1	2.5	13.6	28.6	50.5	66.1	56.5	18.0
7.5	3.5	14.1	29.6	52.1	66.6	57.5	20.0
8.5	3.0	13.0	29.5	52.5	67.0	57.1	13.3

The reaction of the medium had very little effect on the growth of the organism; *Fusarium eumartii* is apparently able to grow well at all H-ion concentrations between a pH of 4.5 and one of 8.5. The slight difference occurring can be seen in Table 2. At the optimum temperature the reaction of the medium had no great effect on growth; if anything the more alkaline medium favored the growth of the organism. At the extreme temperatures, a greater difference was noted. The more acid medium resulted in a considerable decrease in the total growth, and this decrease was greater as the temperature departed from the optimum. The high alkaline set, pH 8.5, did not cause any great decrease in growth except at 33° C. The data presented in Table 2 are the average

of one experiment run in triplicate, with a duplicate experiment conducted at 33° where the greatest differences occurred. From the preliminary evidence presented here, it becomes necessary to take temperature into consideration in experiments to determine the effect of H-ion concentration on the growth of an organism in culture.

The results presented here are in accordance with the available data on the natural distribution of *Fusarium eumartii*, as regards both temperature and soil reaction.

Isolation Studies

An analysis of the results of several thousand isolations made during this investigation reveals several important points regarding the behavior of the causal organism. The isolations were made from all parts of affected plants in the field and others which had been inoculated with pure cultures of *Fusarium eumartii* in the greenhouse.

Many workers have made isolation studies of potato tubers showing various types of internal discoloration. No detailed summary of these investigations need be made other than to state that the results have been variable. Usually a large number of failures to isolate any organism have been reported, the recovery of tuber-rotting species of Fusarium rather than wilt parasites has been very frequent and these were often associated with other miscellaneous fungi and bacteria. The only reported isolations of *Fusarium eumartii* are from Pennsylvania and New York. Carpenter (1) noted that the vascular bundles were often discolored in advance of the stem-end rot, and that in inoculation experiments a water-soaked zone of enzymic activity preceded the rot. Isolations from the border of such discolored bundles or from the watery zone were often negative whereas *F. eumartii* could be obtained from the margin of the discolored tissue and the watery zone. He was unable to isolate *F. eumartii* from one type of stem-end necrosis and he therefore considered this to be distinct from the disease caused by this species. Haskell (8) reported that about 60 per cent of his isolations from affected tubers yielded *F. eumartii*. No reports of the isolation of *F. eumartii* are found in any of the investigations of potato wilt or tuber-rot made in the middle western states by various workers. It is quite possible that potato wilt and vascular infection of potato tubers in many of these states is not identical with the disease that is so common in Nebraska.

However, the following considerations may throw some light upon the investigation of diseases of this type.

During the early part of the investigation the isolations were made from tubers in the usual manner. Affected tubers were washed, treated with mercuric chloride or alcohol, cut with a sterile knife, and then broken so that a small portion of the affected tissue could be removed from the exposed surface. The isolations were always made from the extreme edge of the discolored tissue. The great majority of these isolations proved to be sterile, but often miscellaneous fungi, usually species of Fusarium and bacteria, were obtained. *Fusarium eumartii* was never obtained.

Further isolations were made from tubers, by removing a portion of tissue 6 to 12 mm. back of the extreme edge of the discolored tissue. In these cultures, *Fusarium eumartii* was recovered a number of times but the number of miscellaneous fungi present in such tissue was very great. Isolations were then made from the roots of plants which produced these tubers and these yielded *F. eumartii* in the majority of cases.

Further detailed studies were made by isolations from all parts of plants grown in inoculated soil. These isolations from the roots in nearly every instance yielded *Fusarium eumartii.* The basal stem of affected plants often revealed the causal organism, but those which were badly disintegrated also yielded a variety of fungi. Isolations from discolored pith occurring chiefly at the nodes of the upper part of the stem were always sterile. Affected tubers with the stolons still attached were removed and isolations made immediately. If the tuber was only slightly infected the organism could be recovered from the stolon, whereas many cultures from the tubers were sterile. The organism usually was recovered from tubers in which the disease was further advanced, except from the extreme edges of the discolored tissue which were always sterile. Many tissue cultures made from the extreme edges of the discolored vascular bundles on potato agar showed a deep brown discoloration of the agar after 24 hours, which increased in amount for 3 to 4 days (Plate 5, B), after which diffusion thru the agar became so general that the darkening of the medium around the tissue was not evident. Such cultures usually were sterile. Some affected tubers were separated from the stolons and stored under ordinary storage conditions for a month or more. Isolations from these tubers yielded a variety of organisms, chiefly *F. trichothecioides* and *Alternaria* spp. In many instances *F. eumartii* could not be obtained.

It was evident from these results that *Fusarium eumartii* was capable of producing a toxic substance by which the host tissue was discolored and killed for a considerable distance in advance of the organism. It was also evident that affected tubers were subject to invasion by secondary parasites which grew more rapidly than *F. eumartii* and were thus capable of overgrowing it and preventing its isolation.

These two points serve to explain why so many of our cultures from affected tubers were sterile or yielded a variety of miscellaneous fungi. It was not until these points were ascertained that a clear perspective of the problem in Nebraska was possible. An ordinary cultural study of stem-end rot tubers in storage in western Nebraska would certainly never indicate the real causal factor. However, the isolation of the causal organism from the roots and other parts of affected plants in the field and the positive results so easily obtained in infection experiments established without a doubt the identity of the real pathogene.

Toxic Secretions of the Organism

IN RELATION TO WILT

The examination of sections of infected plants revealed the fact (p. 20) that the vascular elements were not blocked by the growth of the organism thus causing a wilt of the plant, but that the tissue was often killed in advance of the organism. A few preliminary tests were therefore conducted to determine whether or not *Fusarium eumartii* can produce any toxic substances which might cause infected plants to wilt.

Cultures were grown in liquid media.[1] The solution was divided into 300 c. c. portions and placed in 2-liter flasks. The cultures were grown for 3 weeks at 25° C. The fungus mat was then removed and the remaining solution filtered and treated in various ways. The tops of healthy potato plants were cut off and placed in sterile bottles containing the filtrate and placed in the shade in a greenhouse held at 18°. Observations were then made on the wilting phenomena.

The culture solution when inoculated showed a reaction of pH 4.5 and after 3 weeks' growth the solution was found to be much less acid, testing pH 6.5. The treatment of the solution and the effects on wilting were as follows:

[1] 10 gm. KNO_3. 5 gm. KH_2PO_4, 2.5 gm. $MgSO_4$, .02 gm. $FeCl_3$, 50 gm. sucrose, in 1500 c.c. of distilled water.

Treatment	Rate of Wilting
Filtered thru cotton, boiled 15 minutes.	No wilting.
Filtered thru cotton, unboiled.	Slight wilt in 30 minutes, complete wilt in 2½ hours.
Filtered thru Mandler filter, unboiled.	Wilting in 2 to 6 hours.
Sterile culture solution.	No wilting.

It was evident from these few tests that *Fusarium eumartii* is capable of producing a toxic substance which may cause a rapid wilt of potato foliage when placed in the filtrate and which is destroyed by boiling for 15 minutes. It is possible that this toxic substance may be a factor in producing the wilt symptoms in growing plants. Wilting would also be accelerated by the destruction of roots and by the decay of the basal part of the stem. The destruction of the cortex of the roots does not necessarily result in wilt if sufficient soil moisture is available. The destruction of the host tissue may, however, give rise to decomposition products toxic to the plant, as other workers have previously pointed out. It is probable that it is a combination of these factors which causes wilting of infected plants.

The results of somewhat similar tests made by the author in 1915 with *Fusarium oxysporum* have been briefly reported (2). For purposes of comparison the methods used and the results of these tests are presented here in detail. *F. oxysporum* was grown for 3 weeks, the same length of time used in the tests with *F. eumartii,* in a similar medium. In some of these tests the mat of mycelium was removed, washed, ground up, water added, and the solution filtered thru a Berkfeld filter. In others the solution remaining after the removal of the mycelium was filtered thru a Berkfeld filter. The results were as follows:

Treatment	Rate of Wilting
Filtrate from extract of mycelium.	Slight wilt in 3 days, complete in 5 days.
Filtrate from culture.	Wilted in 24 hours.
Filtrate from culture boiled for 10 minutes.	Slight wilt in 24 hours, complete in 48 hours.
Filtrate reduced by boiling to half the initial volume.	Wilt in 12 hours, complete in 36 hours.
Spore suspension in culture medium.	Wilted in 24 hours.
Sterile culture medium.	No wilt.

From these tests it was evident that the toxic substance produced by *Fusarium oxysporum* was not destroyed by boiling and that the action of this substance in causing wilt was very slow.

Young and Bennett (11) have reported more extensive experiments of the same general nature, with *Fusarium oxysporum*. They reported that an increase in the age of the culture resulted in a decrease in the wilting period when potato stems were placed in the filtrate. They reported a slower rate of wilting than that obtained by the author. They also reported "that autoclaving for 15 minutes or boiling for 30 minutes did not alter materially the wilting period of the filtrate."

These tests with *Fusarium eumartii* and *F. osysporum*, while not exactly comparable, differ only slightly in the methods employed and yield results that are strikingly different. The toxic action of the filtrate of cultures of *F. oxysporum* is very slow, and is not destroyed by heating; in fact, the "wilting period" is decreased when the volume of the filtrate is reduced by boiling. The filtrate from cultures of *F. eumartii* results in a rapid wilting of potato stems and the toxic action of this filtrate is destroyed by boiling.

It is significant that the comparative action of these toxic substances produced by *Fusarium eumartii* and *F. oxysporum* can be correlated with the more rapid wilting and the more destructive action of *F. eumartii* when plants are inoculated with pure cultures of the organism.

IN RELATION TO TUBER ROT

As previously noted, *Fusarium eumartii* causes a discoloration of the tissue in the potato tuber for a considerable distance in advance of the organism. A few preliminary tests were therefore made to determine whether or not the organism produced toxic substances in pure culture, capable of destroying healthy potato tissue.

The methods employed in these preliminary tests were somewhat similar to those described by Harter and Weimer (9), and the accuracy of the methods need not be discussed here. Cultures were grown for 7 days in 300 c. c. portions of potato extract at 25° C. The mycelial mat was then removed and the solution filtered thru cotton and divided into 25 c. c. portions which were placed in 125 c. c. flasks. Freshly cut potato disks 15x1.5 mm. in size were placed in the solutions, 5 in each flask, and the flasks incubated at 37.5°. The test was run in triplicate. Maceration of the disks was determined by pulling apart with the fingers.

No maceration occurred in the sterile potato extract in 24 hours. The boiled filtrate gave similar results. Unboiled

filtrate resulted in slight maceration in 2 hours, and complete disintegration in 5 hours. In one test toluol was added to the solutions and the results were the same as those without toluol. Microscopic examination of the macerated tissue revealed the disintegration of the middle lamella and the separation of many of the individual cells.

The action of this toxic excretory substance was not as rapid as that reported for species of Rhizopus by Harter and Weimer (9). The tests reported here were small in number and of a preliminary nature, yet they give some indication of the manner in which *Fusarium eumartii* may kill the host tissue in advance of its invasion by the production of a thermolabile toxic excretion.

GREENHOUSE EXPERIMENTS

Preliminary Pathogenicity Tests

Inoculation by wounding:—Healthy Bliss Triumph plants were inoculated with *Fusarium eumartii* by introducing a small amount of spores and mycelium, with a sterile scalpel, into the stem just below the surface of the ground. Four plants were inoculated with cultures of the organism which had been grown for 2 weeks on hard oat agar.

Seven days after inoculation one plant showed the first symptoms of wilt. Five days later one stem was nearly dead and the foliage of the other stem was seriously wilted. Three days later the plant had completely wilted and was dug up. Examination showed that the organism had produced complete disorganization of the stem at the point of inoculation and had advanced several inches above the point of inoculation and also below it as far as the roots. A second plant showed symptoms of wilt 23 days after inoculation, and 5 days later the other plants developed symptoms. Two weeks later all 3 of these plants were completely wilted and when dug up revealed a similar condition to that described for the first plant. Isolations were made from all plants and the causal organism recovered. Control plants remained healthy thruout the experiment.

These inoculations were all made in the greenhouse at a temperature of about 25° C. At this temperature and with the inoculation methods used, the progress of the disease was more rapid than usually occurs in the field.

Soil inoculations:—Ten pots were filled with soil which had been inoculated with a small amount of material from 2 weeks old rice cultures of *Fusarium eumartii*. They were

then planted with healthy Bliss Triumph seed. These plants did not show any marked symptoms until about 5 weeks after planting when they were growing vigorously. Two weeks later all plants showed typical symptoms of the disease as it occurs in the field. The lower leaves became slightly mottled, that is, the veins and the tissue immediately adjacent to the veins remained green while the rest of the leaf became a light greenish yellow color. Eventually all the lower leaves turned yellow and wilted; at the same time the symptoms began to appear on the younger foliage. In a little over 2 months after planting, all the plants had completely wilted and were dug. Every plant showed root injury and internal discoloration of the stem in varying amounts. Four of these plants produced tubers showing typical stem-end rot. Control plants remained healthy thruout the experiment.

Altho only a few plants were used in these tests, the results were so uniform that it was considered proof of the active pathogenic nature of the organism under consideration. The rapidity with which the organism attacked the host tissue, and the extent of invasion when direct inoculations were made into the stem, indicated a more virulent parasite than the author had previously encountered among the species of Fusarium isolated from potatoes.

In inoculated soil the disease did not progress as rapidly as from stem inoculations. However, the fact that the plants were able to form tubers before complete wilting occurred permitted observations on the new tubers. The presence of typical stem-end rot in the new tubers, in connection with the uniformity of wilt symptoms in all plants, and the similarity in the appearance of these plants to that of the disease in the field indicated that the causal organism in the production of potato wilt and stem-end rot in Nebraska had been obtained.

Infection Experiments with Controlled Soil Temperatures and Soil Moistures

Methods:—In all the following greenhouse experiments the methods used were as follows, unless otherwise noted. The soil (3 parts of soil and 2 parts of sand) was mixed in large enough quantities to provide for all experiments for one year. This soil was steamed for 4 hours at 2½ pounds pressure. Samples were then taken for the determination of the soil moisture equivalent[1]. The moisture content of the soil was determined when the containers were filled, and the moisture content was then adjusted to the desired point.

[1] The author is indebted to Mr. J. C. Russel of the Agronomy Department, Nebraska College of Agriculture, for the determination of soil moisture equivalents which are expressed in this publication by the abbreviation "M. E." with the ratio expressed by the figure prefixed; as, "1¼ M.E."

During the experiments water was added one to three times a week, depending upon the growth of the plant. The water was added by using a small inverted clay pot placed several inches below the surface of the ground for a reservoir, the water being added thru a small glass tube. Enough of the required water was also added to the surface of the soil to keep the moisture content fairly uniform thruout the container. The surface of the soil was covered with 1000 grams of gravel to prevent baking of the surface and to reduce temperature fluctuation in the surface soil.

In the temperature experiments, galvanized iron containers were used which held 10 kilos of soil. In the moisture experiments, 3-gallon earthenware crocks holding 11 kilos of soil were used. The methods were in general similar to those previously described in experiments with *Fusarium oxysporum*, Goss (6), except that improvements were made where possible and the soil moisture contents were expressed on the basis of the moisture equivalent, which was found to be more reliable than the previous method of using the moisture holding capacity as a basis.

Soil inoculations were made by mixing with the soil, cultures of the organism grown on a mixture of rice and cornmeal. About one pint of this inoculum from 3-weeks old cultures was applied to 25 kilos of soil.

In the seed inoculation experiments the cut seed was placed for a few minutes in a spore suspension from 3-weeks old cultures on hard oat agar, and then planted immediately.

Seed pieces of approximately the same size were used thruout the experiments. The seed used for all experiments in any one year was obtained from a lot of healthy Bliss Triumphs, the only potato variety used in this study.

Two greenhouses were employed, one held at approximately 25° and the other at 18° C. Soil temperatures were maintained by the use of modified "Wisconsin soil temperature tanks." These "tanks" were kept in the 18° greenhouse. The soil temperature at a depth of 3—4 inches was recorded 3 times daily.

RELATION OF SOIL TEMPERATURE TO THE DISEASE

SOIL INOCULATION

Experiment 3, December 14, 1922—February 16, 1923

Methods:—The soil used in this experiment was kept at a moisture content of 22 per cent (dry weight) equaling the moisture equivalent. The experiment was conducted in a

greenhouse held at a mean temperature of 18° C. Five soil temperatures were used as follows: 15°, 20°, 25°, 30°, and 35°. Records of the temperatures gave means of 14.8°, 19.7°, 24.7°, 29.8°, and 34.6°. Six inoculated plants and 2 controls were grown at each temperature.

Results:—The results of this experiment are presented briefly in Table 3. The most favorable temperature for infection and for the development of the disease was 20° C., where all inoculated plants wilted completely, (Plate 3, C). The number of plants infected and the severity of the disease decreased as the temperature departed from 20°. At 35° only 4 seed pieces in inoculated soil and one control germinated. Two inoculated and one control wilted completely owing to the extremely high soil temperature. The symptoms were the same in all 3 plants and were not typical of plants infected by *Fusarium eumartii,* which could not be recovered from these plants.

Two inoculated and one control plant grown at 18° (soil and air temperature) and changed 54 days after planting to a soil temperature of 35° with the air temperature remaining at 18° C. yielded very interesting results. The inoculated plants showed symptoms of wilt 24 hours after the change of temperature and wilted completely in 3 days, whereas the control plant, after a slight drooping and flaccidity which occurred immediately after the change of temperature, completely recovered. The quick change to such a high soil temperature undoubtedly disturbed the physiological balance of the plants. The control plant with its healthy root system was evidently able to adjust itself to the new environment. The inoculated plants, which upon digging were found to be severely infected with many of the roots destroyed, were unable to adjust themselves to the new conditions and death resulted.

The symptoms of the disease developed most rapidly at 20° C.; the first symptoms appeared in 11 days (average), and complete wilt occurred in 26 days (average) after emergence. The time increased at the higher and lower temperatures. The difference would be even more marked if the time had been calculated from the date of planting, as emergence occurred in the following order: 25°, 30°, 20°, 15°, while the disease symptoms appeared first at 20°. No differences in the rate of emergence could be noted between the control and inoculated plants. There was considerable dif-

TABLE 3.—*Relation of soil temperature to the disease*
Soil Inoculation
Experiment 3

Soil temperature	No. of plants	Average height	Number of plants and character of symptoms												Relative rate of progress of the disease after emergence			
			Wilt				Root infection			Internal necrosis								
										Basal stem			Upper stem					
			Healthy	Slight	Serious	Complete	Healthy	Slight	Serious	Healthy	Slight	Serious	Healthy	Slight	Serious	Number of plants wilting	Average number of days from emergence to first symptoms	Number of plants completely wilted	Average number of days from emergence to complete wilt
°C.		*Inches*																	
15	6	20 (25)[1]	2	3	1	0	0	4	2	0	4	2	5	1	0	4	31	0	..
20	6	7 (24)	0	0	0	6	0	0	6	0	0	6	0	0	6	6	11	6	26
25	6	12 (18)	0	0	2	4	0	0	6	0	0	6	0	1	5	6	19	4	30
30	6	9 (11)	3	2	1	0	0	4	2	3	1	2	6	0	0	3	31	..	..
35	4[3]	4 (4)	2	0	0	2	No disease appeared												
(18)[2] 35	2	12 (24)	0	0	0	2	0	0	2	0	0	2	1	0	1	2[2]			

[1]Average of control plants in parenthesis.
[2]One control and two inoculated plants grown at 18° C. were placed at 35° on February 7. The tops appeared healthy at time of changing. First symptoms appeared on inoculated plants in 24 hours. Complete wilt in 3 days.
[3]Two seed pieces did not germinate, 2 plants completely wilted due to the high temperature.

ference in the height of the control and inoculated plants, particularly at the optimum temperature for the disease.

The relation of soil temperature to the degree of severity of root infection and internal discoloration of the stem and tops closely paralleled the appearance of the symptoms of wilt.

The control plants in all instances remained healthy except at 35°, where wilting occurred due to high temperature, and in one control at 30° C. which did not show any vascular discoloration of the stem. No causal organism could be isolated from this plant.

Twenty-four isolations were made from the roots of wilted plants grown at 15°, 20°, 25°, and 30° C. and all yielded *Fusarium eumartii.*

Experiment 4, February 17—May 18, 1923

Methods:—In this experiment the same containers and soil used in Experiment 3 were replanted with seed from the same source. No additional soil inoculation was made. The soil moisture was held at 22 per cent (dry weight), as in Experiment 3.

Soil temperatures midway between those used in Experiment 3 were employed. These were 12.5°, 17.5°, 22.5°, 27.5°, and 32° C. The containers used at these temperatures were the same ones used in the previous experiment at 15°, 20°, 25°, and 30° respectively. Those previously held at 35°, where no disease appeared, were transferred to a greenhouse kept at 25° to determine whether or not the organism had been killed or had lost its virulence. Another set of 6 containers was made up as in the previous experiment and held at 32°. Records of the soil temperatures taken 3 times daily averaged as follows: 12.6°, 17.9°, 22.4°, 26.7°, and 31.6°. At the two highest temperatures the average was slightly below the desired point.

The experiment was conducted in a greenhouse held at a temperature of 18° C. Notes were taken thruout the experiment as in Experiment 3, and in addition, data were collected on the infection of the new tubers.

Results:—The results of this experiment are presented in Table 4. The most favorable temperature for the development of the disease was 22.5° C. where all inoculated plants were infected and 5 out of 6 completely wilted. The injury to all parts of the plant including the amount of infection in the new tubers was greatest at this temperature. At temperatures below the optimum, infection occurred in all plants but the disease was not as severe. Above the optimum both

TABLE 4.—*Relation of soil temperature to the disease*
Soil Inoculation
Experiment 4

Soil temperature	No. of plants	Average height	Number of plants and character of symptoms												Effect of disease on tuber production					
			Wilt				Root infection			Internal necrosis						Number of plants producing tubers		Average weight of tubers per plant		Per cent of tubers infected (by weight)	
										Basal stem			Upper stem								
			Healthy	Slight	Serious	Complete	Healthy	Slight	Serious	Healthy	Slight	Serious	Healthy	Slight	Serious	Control	Inoculated	Control	Inoculated	Control	Inoculated
°C.		*Inches*																*Grams*		*Per cent*	
12.5	6	32 (37)[1]	3	3	0	0	0	5	1	4	2	0	6	0	0	1	2	43	35	0	0
17.5	6	15 (24)	2	1	0	3	0	3	3	0	3	3	4	0	2	2	4	125	77	0	37
22.5	6	13 (22)	0	1	0	5	0	1	5	0	1	5	2	3	1	2	6	130	107	0	44
27.5	6	21 (18)	1	0	3	2	1	2	3	1	2	3	6	0	0	2	4	70	69	0	18
32	2	12 (17)	0	1	0	1	1	0	1[2]	1	0	1	1	0	1	..	..	..	..	..	..
18—32[3]	2	20 (26)	0	0	2	0	0	0	2	0	0	2	0	2	0	..	..	..	..	..	..
25[3]	6	19 (21)	0	0	4	2	0	2	4	0	2	4	6	0	0	2	4	60	39	0	90

[1]Two plants previously grown at 18° were changed to 32° C. on April 3.
[2]Isolations from roots of this plant did not yield *Fusarium eumartii*.
[3]Soil and air temperature both 25°.
[4]Average height of control plants in parenthesis.

the amount of infection and the severity of the disease decreased. At the maximum temperature used, 32°, four of the seed pieces failed to germinate. Both plants which developed wilted, altho one was probably due to high temperature, as no trace of infection could be found in the underground portions. Plants started at 18° and changed to 32° showed rather rapid wilting immediately after placing them at the higher temperature. The action here was probably similar to that noted in Experiment 3. In the greenhouse held at 25°, the disease occurred in all inoculated plants and progressed in about the same way as at 22.5°. This soil had been held at 35° during the previous experiment and no disease occurred. It is evident, therefore, that the organism was not killed at that temperature.

Many of the severely infected plants in this experiment failed to produce tubers. The average weight of tubers from inoculated plants was considerably less than from the controls. A high percentage of the progeny of inoculated plants showed typical stem-end rot as illustrated in Plate 1, B, C. The per cent of stem-end rot would be higher if the number of tubers rather than the weight had been used for comparison, as most of the infected tubers were smaller.

Inasmuch as the seed used in this experiment had been in storage longer than that used in Experiment 3, the germination and the early growth of the plants was more rapid. As a result the first symptoms did not appear as early as in the previous experiment, and the disease did not progress as rapidly in the infected plants. Considerable variation in the time of appearance of symptoms occurred in different plants held at the same temperature. This was apparently due to the fact that if infection occurred early or if a plant grew slowly at any temperature above 17.5° C. it wilted much more quickly than one that had managed to develop a good root system before the disease had progressed very far.

The controls in all cases remained healthy except at 32° C. where slight wilting occurred altho the causal organism could not be recovered in culture. Wilting in this case was probably due to the high soil temperature.

SEED INOCULATION

Experiment 5, November 8, 1923—February 11, 1924

Methods:—The soil used in this experiment was steamed in the same way as in previous experiments. The soil moisture equivalent was 15 per cent dry weight. Inasmuch as the soil

TABLE 5.—*Relation of soil temperature to the disease*
Seed Inoculation
Experiment 5

Soil temperature	Number of plants	Average height	Number of plants and character of symptoms																Relative rate of progress of the disease after emergence			
			Wilt				Root infection[1]			Internal necrosis						Seed piece infection				Resulting tubers						
										Basal stem				Upper stem												
			Healthy	Slight	Serious	Complete	Healthy	Slight	Serious	Healthy	Slight	Medium	Complete	Healthy	Slight	Serious	Healthy	Slight	Serious	Complete	Number healthy	Number infected	Number of plants wilting	Average number of days from emergence to first symptoms	Number of plants completely wilted	Average number of days from emergence to complete wilt
°C.		*Inches*																								
12	6	21	6	0	0	0	6	0	0	5	1	0	0	6	0	0	1	3	2	0	19	0	0	..	0	..
16	6	21	1	4	1	0	3	2	1	1	1	2	2	3	1	2	0	1	0	5	10	5	5	25	0	..
20	6	6	0	0	1	5	0	0	6	0	0	5	1	0	0	6	0	0	0	6	0	2	6	19	5	33
24	6	8	0	0	2	4	0	0	6	0	0	2	4	0	1	5	0	0	0	6	0	2	6	12	4	20
28	6	6	0	0	1	5	0	0	6	0	0	0	6	0	0	6	0	0	0	6	0	3	6	18	5	53
32	6		Infected plants and controls appeared the same, complete wilt due to high temperature																							

[1]Root infection at all temperatures started at the base of roots adjoining the infected stems.

contained more sand than that used in previous experiments, the moisture content was kept at 18 per cent (dry weight). Six different soil temperatures were used, 12°, 16°, 20°, 24°, 28°, and 32° C. The air temperature was kept at 15° to 18°. Records of the soil temperatures showed averages of, 11.9°, 15.9°, 23.9°, 27.4°, and 31.3°.

Healthy Bliss Triumph tubers were inoculated by dipping the freshly cut seed in a spore suspension of *Fusarium eumartii*. They were planted immediately and placed at the desired temperature. Six inoculated and 2 control plants were grown at each temperature.

Results:—The notes taken thruout the experiment and the data collected when the plants were dug are presented in Table 5. The relation of soil temperature to infection from the seed is approximately the same as in the previous experiments with soil inoculation. The disease was most severe at temperatures of 20° to 28° C. The wilt symptoms were most rapid at 24°. Slight infection of the basal portion of the stem occurred in one plant at 12°, and the number and severity of stem infections increased with increasing temperature. Discoloration of the pith of the upper stem also occurred more severely at the higher temperatures. Both inoculated and control seed pieces had all rotted at the close of the experiment except at 12°. The plants at 12° and 16° were still growing vigorously at the end of the experiment and the symptoms of the infected plants would probably have been more severe if they had been dug later. Tuber infection occurred at 20°, 24°, and 28°, but the number of tubers was small owing to the early death of the plants.

At 32° all the plants, including the controls, completely wilted, the foliage was chlorotic, severely spotted, and both the leaves and stems were badly streaked; the pith of the stem was disorganized and the basal part of the stem considerably rotted. It was impossible to distinguish between infection and the effects of the high temperature, so no detailed notes are given for this temperature.

The control plants at all other temperatures remained healthy thruout the experiment.

RELATION OF SOIL MOISTURE TO THE DISEASE
SOIL INOCULATION

Experiment 6, December 14, 1922—January 26, 1923

Methods:—This experiment was conducted in a greenhouse having a mean temperature of 23.4°, with a mean maximum of 24.8° and a mean minimum of 23.1° C.

In this experiment 52 three-gallon crocks were used. The methods employed have been described on p. 39. The soil was inoculated in the same way and at the same time as in Experiment 3.

The moisture equivalent of the soil was 21.9 per cent. The experiment comprised 3 sets as follows: One set of 4 controls and 9 inoculated plants, held at 22—20 per cent (dry weight), this being approximately equal to the moisture equivalent; one set of 8 controls and 18 inoculated plants was kept at 27 per cent, or 1¼ M. E.; and one set of 4 controls and 9 inoculated plants was kept at 33 per cent or 1½ M. E.

Results:—The first symptoms of disease appeared 27 days after planting and 9 days after emergence. The next day 16 plants showed symptoms of the disease. The disease at this time progressed very rapidly, the lower leaves wilting and dying within 48 hours after the first symptoms appeared. The first appearance of the disease in this experiment was characterized by the following symptoms noted on a typical plant: ten days after emergence the lower leaf appeared flaccid and after 24 hours showed a yellowing or drying at the basal margins; the petiole turned brown, shrivelled, and hung to the stem by a thread. By this time the leaves higher up began to show the same symptoms. On the third day, the entire tops of infected plants were flaccid and drooping. At this time, chlorotic leaves showed black veins when held against the light, the petiole showed a black vascular system, and if the main stem was held against a strong light a brown flecking was apparent in the pith.

Occasionally leaves with incipient mottling and yellowing appeared similar to affected plants under field conditions but in general the disease progressed more rapidly than usually occurs in the field.

The results of this experiment are presented in Table 6. No effect of the disease could be noted on germination. When the plants were dug on January 26, only 43 days after planting, 100 per cent infection was evident. Only one plant out of the 32 plants grown on inoculated soil failed to show wilt symptoms, and 23 plants had already completely wilted.

The greatest percentage of plants with complete or severe wilt occurred in the set with the highest moisture content. All these plants would undoubtedly have wilted completely if the experiment had been allowed to continue, but the difference between the various sets would not have been as marked. The effect of the higher moisture content was shown in the degree of injury, the disease appearing earliest, the

TABLE 6.—*Relation of soil moisture to the disease*
Soil Inoculation

Experiment 6

Soil moisture[1]	Number of plants	Average height	Number of plants and character of symptoms																Relative rate of progress of the disease after emergence			
			Wilt				Root infection			Internal necrosis						Seed piece infection				Number of plants wilting	Average number of days from emergence to first symptoms	Number of plants completely wilted	Average number of days from emergence to complete wilt
										Basal stem			Upper stem										
			Healthy	Slight	Serious	Complete	Healthy	Slight	Serious	Healthy	Slight	Serious	Healthy	Slight	Serious	Healthy	Slight	Serious	Complete				
		Inches																					
1 M. E.	8	6 (9)[2]	1	1	2	4	0	2	6	0	4	4	1	2	5	0	2	4	2	7	10	4	17
1¼ M. E.	15	10 (20)	0	1	3	11	0	1	14	0	3	12	1	1	13	0	3	8	4	15	8	11	19
1½ M. E.	9	12 (21)	0	0	1	8	0	0	9	0	0	9	0	0	9	0	2	5	2	9	10	8	22

[1]Soil moisture content is expressed as proportion of the moisture equivalent.
[2]Average height of healthy control plants in parenthesis.

progress of the disease being slower but eventually resulting in more complete penetration of all host tissues affected.

An examination of the figures in Table 6 relative to the amount of root infection and seed infection shows very clearly how infection occurred. Out of 36 plants only 8 showed complete rotting of the seed piece, 4 being bacterial rots which prevented germination. Twenty-three out of 32 seed pieces grown in inoculated soil showed healthy tissue around the sprout, and 19 showed vascular discoloration in the seed tubers originating at the base of the stem. There is no positive proof that this infection of the vascular system in the seed piece started in the roots and progressed thru the stem to the seed. However, 8 of the wilted plants showed healthy tissue surrounding the sprout whereas the root systems of all wilted plants were seriously infected, thus indicating strongly that infection occurred almost entirely thru the roots. It was also clear that all seed pieces became infected from the soil, but the rapidity of the action of the root infection caused death of the plant before the seed piece infection could reach the stem, (Plate 7, A).

Practically all of the inoculated plants were dead before tuber formation occurred. All control plants remained healthy thruout the experiment.

Experiment 7, February 5—May 25, 1923

This experiment was planned to obtain data on the effect of both temperature and soil moisture on the development of the disease.

Methods:—The containers and the same soil used in the previous experiment were replanted with tubers from the same source. The soil moisture content, therefore, was at the desired point before planting, with the exception of the set at the lowest moisture content which was allowed to dry out to the desired point before beginning the experiment. Soil moisture contents of 2/3, 1, 1¼ and 1½ M. E. were employed. In addition each set of 9 containers at each moisture content was divided into 2 lots, one lot of 5 being placed in a greenhouse maintained at a mean temperature of 23.6° C. The other set of 4 containers was placed in a greenhouse held at a mean temperature of 19°.

Because of the more rapid action of the disease at the higher temperature, these plants were all dug on May 2, while those in the 19° C. house were left until May 25.

Soil Inoculation

Experiment 7

Soil moisture[1]	Temperature	Number of plants	Average height	Number of plants and character of symptoms												Relative rate of progress of the disease after emergence	
				Wilt				Root infection			Internal necrosis: Basal stem			Internal necrosis: Upper stem				
				Healthy	Slight	Serious	Complete	Healthy	Slight	Serious	Healthy	Slight	Serious	Healthy	Slight	Serious	Number of plants wilting	Average number of days from emergence to first symptoms
	°C.		*Inches*															
2/3 M. E.	19	4	23 (26)[2]	2	1	1	0	0	3	1	0	2	2	3	0	1	2	44
	23.5	3	15-1[3] (22)	0	0	1	2	0	1	2	0	1	2	1	0	2	1	32
1 M. E.	19	4	19 (25)	2	2	0	0	0	3	1	3	1	0	4	0	0	2	78
	23.5	5	17 (28)	1	0	0	4	1	2	2	1	2	2	5	0	0	5	35
1¼ M. E.	19	4	24 (21)	0	4	0	0	0	1	3	0	3	1	4	0	0	5	58
	23.5	5	28 (36)	0	1	2	2	0	3	2	1	2	2	4	1	0	5	48
1½ M. E.	19	4	24 (24)	1	3	0	0	0	3	1	1	2	1	3	1	0	3	72
	23.5	5	39 (38)	0	1	3	1	0	2	3	0	2	3	3	2	0	5	35

[1]Soil moisture content is expressed as proportion of the moisture equivalent.
[2]Average height of healthy controls in parenthesis.
[3]Two plants were infected from the seed piece which had rotted when the sprouts were 1 inch high. The entire sprout and roots were killed. Two other seed pieces in this set failed to sprout.

Results:—The results of this experiment presented in Table 7 showed much the same relation to Experiment 6 as all the other experiments conducted at this time showed to the other tests run earlier in the year. The older seed, combined with better sunlight conditions, appeared to favor the growth of the host plant and to delay the appearance of disease symptoms. During the latter part of the experiment, the air temperatures in both houses were considerably above the desired points.

The first plants to appear above ground were at the higher soil moisture contents and they were considerably earlier at the higher temperature. The earliest appearance of the disease was at 23° C. in the set with the highest moisture content. After the first appearance of the disease, the wilt progressed most rapidly in the set with the soil moisture equal to the moisture equivalent. The amount of root and stem injury, however, was greater at the higher soil moisture contents, but the large amount of available water prevented rapid wilting of these plants.

At 19° C. the disease first appeared in the set with a moisture content of 1¼ M. E. The greatest amount of disease occurred in this set as judged by both the above and below-ground symptoms, and also by the number of infected new tubers. With a moisture content of 1½ M.E. the resulting tubers showed more extensive stem-end rotting than at the lower moisture contents. The relative rate of progress of the disease at this temperature was difficult to determine, owing to the late appearance of the symptoms. In general, the plants were much healthier and more vigorous at this than at the higher temperature.

All control plants in this experiment remained healthy. *Fusarium eumartii* was repeatedly isolated from infected roots of diseased plants.

SEED INOCULATION

Experiment 8, December 14, 1922—February 14, 1923

Methods:—In this experiment steamed, uninoculated soil was used. Freshly cut seed pieces from the same source as used in the previous experiments were dipped in a water suspension of spores of *Fusarium eumartii* just before planting. The experiment comprised 4 sets, each having 7 inoculated and 2 control plants. The same 4 soil moisture contents were used as in the previous experiment, being 2/3, 1, 1¼, and 1½

TABLE 8.—*Relation of soil moisture to the disease*
Seed Inoculation

Experiment 8

Soil moisture[1]	Number of plants	Average height	Number of plants and character of symptoms														Relative rate of progress of the disease after emergence		
			Wilt				Root[2] infection			Rot of basal stem[3]			Internal necrosis						
													Basal stem			Upper stem			
			Healthy	Slight	Serious	Complete	Healthy	Slight	Serious	Healthy	Slight	Serious	Healthy	Slight	Serious	Healthy	Slight	Serious	Average number of days from emergence to first symptoms	Number of plants completely wilted	Average number of days from emergence to complete wilt
		Inches																			
1 M. E. to 2/3 M. E.	7	10 (5)[4]	0	0	2	5	0	3	4	0	4	3	0	3	4	5	1	1	19	5	33
1 M. E.	7	12 (18)	0	1	2	4	0	4	3	0	3	4	0	1	6	4	0	3	19	4	60
1¼ M. E.	7	21 (22)	0	2	4	1	0	5	2	0	4	3	0	3	4	4	3	0	21	1	40
1½ M. E.	7	25 (26)	0	2	4	1	0	3	4	0	4	3	0	2	5	2	2	3	23	1	41

[1]Soil moisture content is expressed as proportion of the moisture equivalent.
[2]Root injury was confined to the first roots originating at the base of the stem, and infection progressed from the base towards the tip of the root. No root injury occurred on roots originating above the stem injury.
[3]All inoculated seed pieces were completely rotted; all control seed pieces remained healthy.
[4]Average height of healthy controls in parenthesis.

M. E. The moisture equivalent was 21.9 per cent (dry weight).

The set at the lowest soil moisture content, however, differed from previous experiments in that the experiment was started with a water content equal to the moisture equivalent and allowed to dry out during growth to a moisture content of 2/3 M. E. The experiment was conducted in the same greenhouse as the previous experiment which had a mean temperature of 23.6° C.

Results:—The results are shown in Table 8. The disease progressed much more slowly in this experiment than in the experiment with inoculated soil which was conducted at the same time (compare Table 6 with 8). The slow advance of the disease resulted in foliage symptoms similar to the aspect of the disease in the field. The first symptoms did not appear until about 20 days after emergence as compared with about 10 days for soil inoculations in Experiment 6. A greater number of plants completely wilted in the set started at 1 M. E. and allowed to dry out to 2/3 M. E., thus showing that after infection occurred at a favorable soil moisture, the wilting symptoms were accelerated by a drop in the soil moisture content. One hundred per cent germination and infection were obtained in this experiment.

Examination of each plant at digging time showed that the seed pieces were all rotted and that the disease had progressed up the stem (Plate 7, B). The actual amount of rotting of the stem did not vary greatly at the different soil moisture contents. It was found that all roots above the infected area of the stem were healthy whereas those at the base of the main stem were all infected, the infection progressing from the base of the root out towards the tip, the exact reverse of the method of infection in the soil inoculation experiments.

With the higher soil moisture contents, the larger root systems were able to counteract the effects of the disease and keep the tops alive for a considerable length of time altho the infection in the stem was often as great as at the lower moisture contents at which the plants wilted more quickly.

All control plants remained healthy thruout the experiment.

Experiment 9, January 24—May 2, 1923

Methods:—The moisture equivalent of the soil used in this experiment was 18.2 per cent (dry weight). The soil

was allowed to dry out to 2/3 M. E. before planting, and the various sets were brought up to the desired moisture content just previous to planting. The methods used were the same as in the previous experiment, and the seed used was from the same source.

Forty-eight plants were used in this experiment and they were divided into 2 sets, one set being kept in a house held at a mean temperature of 23.6° and the other in a house held at 18.3° C. During the last two weeks of the experiment the temperature during the middle of the day was considerably above the average point desired.

At each temperature the experiment was divided into 4 sets held at moisture contents of 2/3, 1, 1¼, and 1½ M. E. Four inoculated plants and one control were grown at each moisture content and at each temperature.

Results:—The results of this experiment are presented briefly in Table 9. The progress of the disease was much slower than in the previous experiment, conducted in the same manner, as has been noted for other experiments conducted at the same time. The slower progress of the disease resulted in the development of symptoms very similar to the average symptoms found under field conditions. The first symptoms at the higher temperature appeared in the set held at 1½ M. E.; however, complete wilt occurred latest at this moisture content. The most rapid wilting occurred at the low moisture contents. At 18° C. the appearance and development of the disease were much slower. The first symptoms appeared at a moisture content of 1 M. E. altho in this set no complete wilting occurred.

As regards the resulting tubers, the greatest infection occurred at the low moisture contents where very small tubers were produced. At the high moisture contents the yields were greater and fewer tubers were infected. The controls gave 2 to 5 times as large yields as inoculated plants at each moisture content.

One hundred per cent infection was obtained at all moisture contents and at each temperature, the controls remaining healthy in every instance. The amount of infection and the development of the disease in the different sets were approximately the same as in previous experiments.

TABLE 9.—*Relation of soil moisture to the disease*
Seed Inoculation
Experiment 9

Temperature	Soil moisture[1]	Number of plants	Average height	Number of plants and character of symptoms														Relative rate of progress of the disease after emergence			
				Wilt				Root[2] infection			Rot of basal stem			Internal necrosis								
														Basal stem			Upper stem						
				Healthy	Slight	Serious	Complete	Healthy	Slight	Serious	Healthy	Slight	Serious	Healthy	Slight	Serious	Healthy	Slight	Serious	Number of plants wilting	Average number of days from emergence to first symptoms	Number of plants completely wilted	Average number of days from emergence to complete wilt
°C.			*Inches*																				
23.6	2/3 M. E.	4	3 (24)[4]	0	0	1	3	1	0	3	0	0	4	0	0	4	0	0	4	4	14	3	19
	1 M. E.	4	12 (26)	0	0	1	3	0	1	3	1	2	1	0	0	4	0	0	4	4	15	3	39
	1¼ M. E.	5	16 (28)	0	0	1	4	1	2	2	0	2	3	0	0	5	1	1	3	5	13	3	59
	1½ M. E.	5	19 (30)	0	0	3	2	0	1	4	0	3	2	0	0	5	2	3	0	5	17	2 (1)[3]	35 (15)
18.3	2/3 M. E.	5	6 (14)	0	0	2	3	0	3	2	0	2	3	0	0	5	1	3	1	5	29	3	55
	1 M. E.	5	11 (24)	0	3	2	0	0	3	2	0	0	5	0	0	5	0	1	4	5	18	0	..
	1¼ M. E.	5	17 (24)	0	0	2	3	1	2	2	0	1	4	0	0	5	2	2	1	5	33	3	79
	1½ M. E.	5	19 (24)	0	0	2	3	0	2	3	1	0	4	0	2	3	4	1	0	5	36	3	84

[1]Soil moisture content is expressed as proportion of the moisture equivalent.
[2]In all cases root infection had proceeded from the stem, the primary infection coming from the seed piece.
[3]Infection took place at eye instead of thru cut surface of seed.
[4]Average height of healthy controls in parenthesis.
NOTE—In several cases the infected plants died when 3—6 inches high and it was found that infection had taken place in the tissue around the sprout, rather than thru the cut surface of the seed tuber.

Thansmission of the Disease by Stem-End-Rot Seed Tubers

Experiment 10, March 8—May 15, 1922

During the winter of 1921-22, a number of potatoes were secured from western Nebraska showing varying degrees of stem-end rot and various forms of dry rot. A sample consisting of 42 tubers was selected to determine whether or not the disease could be transmitted by such seed.

Methods:—From half of each of the 42 tubers, isolations were made in the usual way. The other half of each tuber was planted in a greenhouse kept at about 25° C. A number of seed pieces were at least half rotted.

Results—Isolation Studies:—All the tubers examined showed a sunken diseased area at the stem-end. The internal appearance varied somewhat, and the tubers were classified in different groups. These groups with the results of the isolations are shown in Table 10. Cultures were made from the extreme ends of the discolored vascular system, or from the edge of the rotted tissue. A large number of the cultures were sterile; many yielded miscellaneous species of Fusarium. Only 4 tubers revealed the presence of *Fusarium eumartii* and 3 yielded *F. oxysporum*. No correlation could be made between the species isolated and the type of injury occurring in the tubers. The most noteworthy feature was the large number of sterile cultures. These cultures were made from the extreme end of the rotted tissue, and later experiments have shown that such tissue is usually sterile when the disease has been produced by *F. eumartii* as discussed on p. 33.

Greenhouse tests:—No typical wilting was observed in any of the plants grown from these badly rotted tubers. The plants in this test were dug at the end of about 9 weeks, and it may have been that wilting would have occurred had it been possible to continue the experiment. The results are shown in Table 10. Plants listed as diseased are those in which rotting or vascular discoloration of the stem occurred. While the number of plants was very small, it can be noted that 3 out of 4 plants yielding *Fusarium eumartii* were diseased and that none of the 3 plants yielding *F. oxysporum* showed evidences of infection. The number of diseased plants was small from those seed tubers yielding no organism in culture. As in the cultural studies the variation in results and the small number of tubers prevent any clear-cut distinctions between the type of injury in the seed and the resulting disease.

TABLE 10.—*Transmission of the disease by stem-end-rot seed tubers*

Experiment 10

Type of seed piece injury	Organisms isolated from seed pieces											
	Sterile		*F. eumartii*		*F. oxy.*		*F. sp.*		Misc.		Total	
	Resulting plants											
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
1. Stem-end rot with vascular discoloration												
a. with rotting of adjacent tissue	8	0	0	1			4	1	3	0	15	2
b. with no rotting of adjacent tissue	3	3					2	2	2	0	7	5
c. with black necrotic strands not confined to vascular system	2	0	1	0	2	0	1	1			6	1
2. Severe stem-end rot with no vascular discoloration	1	1	0	2	1	0	1	0			3	3
Total	14	4	1	3	3	0	8	4	5	0	31	11

Experiment 11, November 14, 1923—February 14, 1924

During the winter of 1923-1924, a number of tubers obtained from the 1923 field experiments (Exp. 14 and 15) were grown in the greenhouse to study the effect of stem-end rot seed on the occurrence of the disease. The type of infection was determined for each tuber, they were then planted in soil which had been steamed for 4 hours at 2 pounds pressure, and were grown in 8-inch clay pots in a greenhouse maintained at a temperature of about 25° C.

The results are presented in Table 11. No correlation could be made between the types of stem-end rot and vascular discoloration in the seed piece and the resulting disease. Three seed pieces failed to sprout. Only about one-half of the plants grown from infected or rotted seed pieces showed any symptoms of disease. Only one plant wilted completely

and more plants were infected at the base of the stem than were indicated by the above-ground symptoms. About 20 per cent of the tubers produced showed evidences of stem-end rot. *Fusarium eumartii* was isolated from a number of the infected stems and tubers. The control plants remained healthy thruout the experiment.

TABLE 11.—*Transmission of the disease by stem-end-rot seed*

Experiment 11

Number of seed	Number germinated	Number of plants with wilt				No. of plants with infection of basal stem				Number of infected tubers	Number of healthy tubers	Number of plants barren
		Healthy	Slight	Serious	Complete	Healthy	Slight	Medium	Severe			
Stem-end rot 60	57	38	11	7	1	29	9	3	16	19	71	9
Controls 15	15	15	0	0	0	15	0	0	0	0	37	9

The majority of the seed pieces planted in this experiment were rather severely infected, most of them being the same type as that shown in Plate 1, D. The resulting disease was not as great nor as severe as would usually be expected from seed of this type. Evidently the primary parasite was either absent or overgrown by secondary invaders in many of the seed tubers showing stem-end rot.

Method and Effects of Infection from the Soil

Method of infection:—While previous experiments have clearly shown that infection can take place from the soil thru the roots, the following test was conducted in such a way that it was possible to observe the progress of this type of infection.

Large glass cylinders were half filled with sterilized sand which was then well moistened with sterile Pfeffer's solution. Healthy Bliss Triumph seed was planted and then a spore suspension of *Fusarium eumartii* was added. The seed sprouted and established a well-developed root system in two weeks. Many of the roots could be observed thru the walls of the

cylinder. The lower part of the cylinder was covered with black paper except when observations were made. Three weeks after planting, the tips of many of the smaller roots were observed to be infected. This appeared as a progressive browning of the part infected, and the killing of all the root hairs. Ten days later the tops of the plants were wilted and the vascular system and pith were discolored for a considerable distance into the tops. When dug, the root system of each plant was found to be seriously infected, and many of the small roots were entirely destroyed. Plate 5, A, shows the appearance of the roots by means of a photograph taken thru the glass wall of the cylinder during the latter part of the experiment. *F. eumartii* was recovered from these roots.

Effect of root infection from the soil:—In many of the experiments reported with soil inoculations it was found that while the seed piece of wilted plants was only slightly rotted from the cut surface, there was very often a discoloration of the vascular system from the sprout back into the seed piece, sometimes reaching the rotted surface (Plate 7, A), and sometimes apparently separated from it by healthy tissue.

In order to determine whether this development of vascular discoloration of seed pieces after planting was the result of infection thru the seed piece or the roots, the following tests were made:

Healthy Bliss Triumph seed was sprouted in sterile sand. When the sprouts were 2 inches high and a good root system had developed, the sand was carefully washed off and they were planted in inoculated soil. The seed piece, however, was not allowed to come in contact with the soil but was separated by placing it in a small clay pot, with the sprout and roots projecting thru the small opening and fixed in place with grafting wax. Sixteen days after planting, all the plants showed symptoms of wilt and 5 days later were completely dead. When dug the roots were found to be almost entirely destroyed and the seed piece had remained sound. There was present, however, a vascular discoloration extending from the roots up the stem into the tops and down the stem into the seed piece.

Further tests were made as follows: Healthy seed pieces were sprouted in sterile sand. When the roots were 3 to 4 inches long, the seed pieces were transplanted into sterile soil in 12-inch pots, which were divided by a board running from the bottom of the pot to about 1 inch above the surface of the soil. A close fit with the sides of the pot was obtained,

and in this way the soil on each side of the board was kept separate. A small hole was cut thru the board several inches below the surface of the soil. The seed piece was planted close to this hole and 2 or 3 of the small roots were drawn thru the hole into the other half of the pot. In various tests these roots were inoculated either by placing a small portion of an agar culture of *Fusarium eumartii* directly on the roots, by inoculating the soil around these roots, or by placing the roots in a test tube containing a spore suspension of the organism. After inoculation of the roots, the soil was placed over them so that growth could continue.

The results were practically the same with each method of inoculation. The inoculated roots became infected, the organism probably reached the stem and the effects of its presence were evidenced by necrosis and discoloration of the tissues not only in an upward direction but also downward to the base of the stem, resulting in a browning of the vascular tissue in the otherwise healthy seed piece. Plate 6, A, shows an example of one of these plants in which wilting of the leaves is apparent. Infection of the roots and seed piece is shown in Plate 6, B. Undoubtedly the observation in the field of such a seed piece would lead one to the erroneous conclusion that infection had occurred thru the seed piece.

These tests showed that a small amount of inoculum applied to only a few roots was capable of producing the typical disease. It was also shown conclusively that it is possible to produce a vascular discoloration in a healthy seed piece as a result of root infection, thus pointing out the unreliability of casual observations made in the field to determine the method of infection.

Discussion of Greenhouse Experiments

Preliminary pathogenicity tests with *Fusarium eumartii* showed it to be a very virulent parasite causing a wilt of the plant and a stem-end rot of the tuber. Infection was obtained by both soil and seed inoculations.

The relation of soil temperature to the disease was determined under controlled conditions. Both soil and seed inoculations resulted in the appearance of the disease; the greatest amount and severity occurred at soil temperatures between 20° and 25° C. At temperatures above and below this optimum range, the number of infections and the severity of the symptoms decreased. A slight amount of infection occurred at a temperature as low as 12°. The root injury at

the low temperatures was greater than was indicated by the wilt symptoms. Considerable infection occurred as high as 30° but infection could not be determined above 30°. At 32° and 35° sprouting was poor, growth was abnormal, and wilting and sometimes vascular discoloration and root injury occurred due to the high soil temperatures. Plants which were infected at a low temperature wilted very rapidly when transferred to soil temperatures above 30°. The infection of the new tubers did not indicate the real damage caused by this disease, as most of the seriously infected plants failed to produce tubers.

The relation of soil moisture to the disease was also determined under controlled conditions and showed the same relative results for both soil and seed inoculations. The disease progressed more rapidly, however, with soil inoculations.

The disease always appeared earliest with the high moisture contents where the wilt symptoms advanced slowly but the disease eventually resulted in more complete penetration of all host tissues affected, including the new tubers. After the first appearance of the disease, wilt progressed most rapidly at the lowest moisture content. Apparently at high moisture contents rapid wilting of the plant is prevented by the abundance of soil moisture, even when the roots are badly decayed, (Plate 4). Usually at these high moisture contents the disease progresses so slowly that new tubers are usually formed which in every instance show stem-end rot. After infection occurred at high moisture contents a sudden drop in the moisture content accelerated the wilt.

The relation of soil moisture to the disease was the same at a temperature of 18° as at 25° C. The disease always appeared first, however, at a higher temperature.

In all the soil moisture experiments 100 per cent infection was obtained. The host relation to the environmental conditions apparently determined the progress of the disease in all these tests. This was not entirely true, however, in the soil temperature experiments, where the progress of the disease was greatly affected by the growth-temperature relations of the pathogene. This was shown by the severity of the disease at the optimum temperature for the growth of the organism, and also by the maximum and minimum temperatures for the disease which closely approximate those for the pathogene.

A detailed examination of all plants, including the seed pieces, in both the moisture and temperature experiments showed that soil inoculation resulted almost entirely in root

infection. Inoculation of seed pieces caused them to rot, and resulted in the progression of the disease up the stem and sometimes out into the roots. In such cases, however, the base of the roots was always heavily infected, while with soil inoculations the tips of the roots were the first to show infection.

In all these experiments it was found that when seed had been stored for some time before planting, sprouting was more rapid, the plant made a better start, and the first disease symptoms appeared considerably later than when the seed had not fully completed its rest period. As a result, seed stored the longest gave disease symptoms very similar to those appearing in the field.

Experiments with stem-end rot seed showed that the type of injury in the seed piece could not be correlated with the kind of organism isolated nor with the disease which developed in the plant. Wilt symptoms in all cases were very slight. About 50 per cent of the plants grown from such stem-end rot tubers remained healthy thruout the experiment. It was apparent, however, that the disease could be transmitted in this manner.

Tests were made to determine more accurately the manner of infection from inoculated soil. It was found that infection could take place thru the roots and that as a result of such root infection the seed piece could become infected by the downward progress of the disease in the stem. It is thus evident that casual examination of the seed piece in the field is not always a good method of determining the manner of infection, as one or two root infections are sufficient to produce symptoms of the disease in both the plant and the seed piece.

When the results of these experiments are compared with those of similar tests previously reported (6) with *Fusarium oxysporum*, it is at once evident that *F. eumartii* is the more virulent parasite and that the experimental results are more in accord with the appearance of the disease in the field. Wilt could be produced with *F. oxysporum* only at temperatures of 30° and above, which made this symptom an unsatisfactory criterion in determining infection, as such temperatures result in a very abnormal growth of the potato plant. The number of infections resulting with *F. oxysporum* were very small as compared with these experiments with *F. eumartii* which usually resulted in 100 per cent infection except under very unfavorable temperatures for the disease. The progress all conditions.
of the disease was always more rapid with *F. eumartii* under

FIELD EXPERIMENTS

Seed Inoculation

Experiment 12, 1921

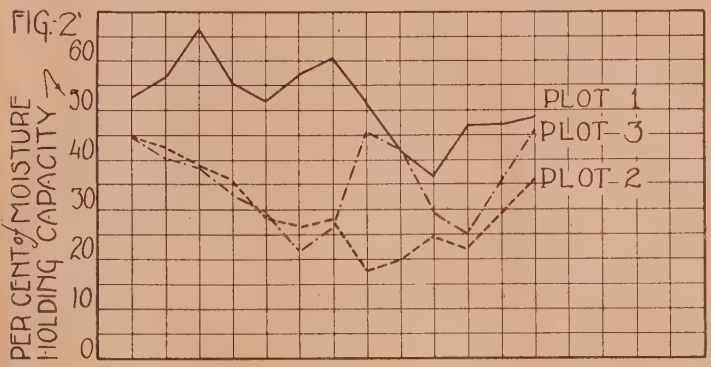
In this experiment inoculated seed was planted at 3 different locations in Nebraska to determine the effect of environment on the disease under field conditions.

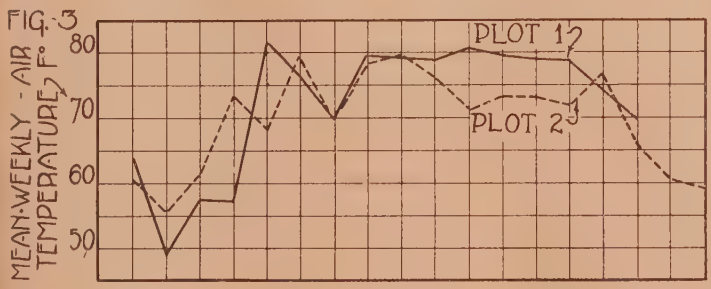
Methods:—Healthy Bliss Triumphs were used for both inoculated and control rows. Inoculations were made by 3 different methods to determine the effect of such methods of infection. Spore suspensions in water from 14-day-old cultures of *Fusarium eumartii* were used for the inoculum. The first method of inoculation consisted in boring a hole in the seed tuber 1 to 1.5 cm. deep and 3 mm. in diameter, in which were placed small glass tubes containing 0.5 c.c. of the spore suspension. In the second method the tubers were inoculated by immersing the freshly cut seed pieces in a spore suspension just before planting. The third method was similar except that the seed pieces were allowed to callus over for 3 days before inoculating. The 3 different experimental plots used and their locations were as follows:

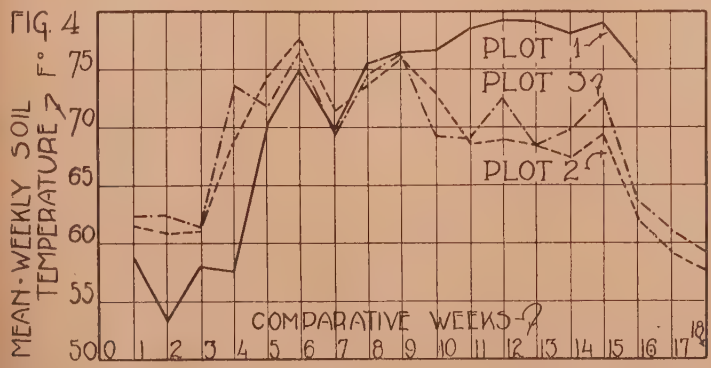
Plot 1—Agricultural College, Lincoln, Nebraska.
Plot 2—Substation, Mitchell, Nebraska, dry land culture.
Plot 3—Substation, Mitchell, Nebraska, irrigated culture.

Plot 1 was planted April 20 and dug August 3 to 8. Plots 2 and 3 were planted May 20 and dug September 21. Figures 2, 3 and 4 represent graphically the comparative mean weekly soil temperature, air temperature, and soil moisture expressed as per cent of the moisture holding capacity of the soil for all 3 plots. Further details regarding these plots have been previously published (6, p. 70-72).

Results:—Table 12 contains a summary of the results of this experiment. In this table all averages and percentages are calculated on the basis of plants rather than upon the number of seed pieces planted. The differences would be still greater between the inoculated and control rows if the number of seed pieces was used as the basis of comparison. No allowance has been made in the yield records for the lack of competition when hills were missing. Again the differences in yields would be even greater if this were considered. Considerable variation is evident and in the main can be correlated with the methods of inoculation and the conditions under whch the experiment was conducted, as shown in figure 2.







FIGS. 2, 3, and 4.—Graphs showing soil moisture (Fig. 2) for 1-foot depths expressed as per cent of the moisture holding capacity, mean weekly air temperature (Fig. 3) and soil temperature (Fig. 4) for comparative weeks of the 3 plots in Experiment 12.

Germination was decreased by a rotting of the inoculated seed pieces. Very little injury occurred in Plot 1, the only evident decrease resulting from the second method of inoculation. In Plots 2 and 3, the germination of the controls was very poor, but this method of inoculation still resulted in a considerable reduction in stand. The soil temperature in Plot 1 was 5° to 10° below that of Plots 2 and 3 during the first 2 weeks of the experiment and the amount of seed piece decay was considerably less. It was also evident that dipping the freshly cut seed in a spore suspension results in the most rapid progress of the disease.

Very little wilting occurred in Plot 1, while it was very severe in Plot 2. Considerable wilting also occurred in Plot 3. The figures in Table 12 represent plants which completely wilted. The wilting can be correlated with the lower soil moisture and temperature occurring in Plots 2 and 3. It is true that the soil and air temperatures in Plot 1 were higher during the latter part of the experiment and it might be expected that wilting would be more rapid. However, the temperature of the soil in Plot 1 during the latter half of the experiment was slightly above the optimum for the disease as previously determined under control conditions, while in Plots 2 and 3, during the period from the ninth to the fifteenth week when wilting occurred, the temperature was at the optimum for development of the disease. The soil moisture content in Plots 2 and 3 was low enough to accelerate the symptoms of wilt. No great difference in the amount of wilt occurred as a result of the different methods of inoculation.

A considerable number of affected plants failed to produce any tubers. Many of these plants did not show severe wilting in the field. The early progress of the disease resulted in a weak spindling plant which did not require much soil moisture to prevent wilting. The greatest number of barren plants was found in Plot 3, even tho the cultural conditions were the most favorable for tuber production as evidenced by the high yield of the control rows. No differences could be noted as being due to the method of inoculation. It was evident that while the first method with the small amount of inoculum used did not cause any great reduction in germination, the organism was capable eventually of rotting the seed piece and advancing up the stem and decreasing tuber production.

The serious effect of the disease was clearly evident when the progeny of the inoculated and control plants was com-

Experiment 12—1921

Plot	Method[1] of treatment	Number of seed planted	Germination	Plants wilted	Plants barren	Plants with infected tubers	Average weight of all tubers per plant	Weight of infected tubers	Yield based on controls as 100 per cent	
									Total yield	Marketable tubers
			Per cent	*Per cent*	*Per cent*	*Per cent*	*Grams*	*Per cent*	*Per cent*	*Per cent*
Plot 1	Inoc. 1	36	97	3.	20.	75.7	51.0	62.0	27.3	11.2
	Inoc. 2	36	89	0.	47.	40.6	16.0	66.0	8.5	2.8
	Inoc. 3	37	100	3.	3.	94.6	153.0	85.0	81.8	11.8
	Control	217	97	0.	2.	10.3	187.0	5.0	100.0	100.0
Plot 2	Inoc. 1	33	94	74.	6.	90.3	111.0	90.0	29.7	2.8
	Inoc. 2	33	33	100.	27.	72.0	43.0	86.0	11.5	1.6
	Inoc. 3	33	42	86.	29.	64.3	63.0	75.0	16.8	4.5
	Control	220	62	0.	1.	12.5	374.0	5.0	100.0	100.0
Plot 3	Inoc. 1	33	91	46.	40.	50.0	155.0	56.0	15.9	6.8
	Inoc. 2	33	21	57.	57.	14.3	117.0	16.0	12.0	10.2
	Inoc. 3	33	66	67.	62.	28.6	207.0	66.0	21.6	7.2
	Control	219	66	0.	1.	1.4	973.0	0.5	100.0	100.0

[1]Inoculation 1. Made by inserting into seed piece a glass tube containing .5 c.c. spore suspension.
Inoculation 2. Made by immersing freshly cut tubers in a spore suspension.
Inoculation 3. Made by immersing tubers in spore suspension after allowing 3 days for the cut surface to heal.

pared. The total weight of all tubers produced by the inoculated plants was with one exception less than 30 per cent of that produced by the control plants. This reduction in yield was partly caused by the number of barren plants. In addition many of the tubers were infected with stem-end rot and showed vascular discoloration. With only one exception, more than 50 per cent of the tubers from inoculated plants were infected while the controls averaged less than 5 per cent infection. If the yields of healthy tubers from inoculated plants be compared, using the controls as a basis, the differences would be even more evident. The inoculated rows in all plots yielded only about 8 per cent of the quantity of healthy tubers produced by the controls.

Plot 1 produced the lowest yields, with increasing yields occurring in Plots 2 and 3 in order. The greatest per cent of infected tubers occurred in Plot 2; no consistent difference in the amount of infected tubers produced by the various methods of inoculation was evident in this plot. A considerable decrease in total yield was noticeable, however, with the second method of inoculation.

Comparison of Stem-End-Rot Seed and Inoculated Seed

Experiment 13, 1922

Previous experiments in the greenhouse have shown the possibility of the disease being transmitted by stem-end rot seed. The results, however, were not as conclusive as the greenhouse and field experiments with inoculated seed. The following experiment was conducted to obtain further data on the transmission of the disease by the seed tubers.

Methods:—Healthy Bliss Triumph seed was used as controls. Some of this same seed was inoculated by dipping the freshly cut seed in a spore suspension of *Fusarium eumartii*. The stem-end rot seed was from the same lot as that used in the greenhouse, Experiment 10, in which infection was of various degrees of severity. They were roughly divided into two lots; slight infection and severe infection, consisting of 135 tubers each. In addition, each tuber was halved lengthwise, one-half was planted and the other half retained for detailed notes on the type of disease and for isolation studies.

The experiment was conducted at the Agricultural College, Lincoln. The seed was planted on May 5 and dug on August 21. The mean weekly air temperature and soil moisture content for the period of the experiment were as follows:

Date	Air Temperature °F.	Date	Soil Moisture[1] Per cent
May 5—May 12	70.1	May 28	57.8
May 12—May 19	58.3	June 5	40.4
May 19—May 26	64.2	June 12	39.4
May 26—June 2	64.8	June 19	37.6
June 2—June 9	74.0	June 26	40.2
June 9—June 16	81.1	July 3	38.6
June 16—June 23	76.3	July 10	45.8
June 23—June 27	76.0	July 17	51.2
[2]July 3—July 10	71.8	July 31	33.2
July 10—July 17	73.6	Aug. 7	32.2
July 17—July 24	73.5	Aug. 14	24.8
July 24—July 31	77.7		
July 31—Aug. 7	78.4		
Aug. 7—Aug. 14	77.5		
Aug. 14—Aug. 21	82.3		

Results:—The stem-end rot seed was divided into groups somewhat similar to those recognized in Experiment 10, and an attempt was made to correlate these different types of injury with the kind of organisms isolated. It was found impossible to correlate the types of infection with the presence of any specific organism, and the detailed results will not be presented here. Neither was it possible to correlate the type of infection or the organism isolated with the resulting disease in the field. The cultures yielded *Fusarium eumartii* from only 4 per cent of the tubers, *F. oxysporum* from 4 per cent, *Fusarium* species, chiefly *F. trichothecioides*, from 32 per cent, miscellaneous fungi, chiefly Alternaria, from 19 per cent, bacteria from 45 per cent, and 11 per cent of the cultures were sterile. Usually several organisms were isolated from the same tuber. The tubers yielding *F. eumartii* gave rise to 60 per cent infection of the plants in the field, whereas all other tubers yielding miscellaneous fungi and *Fusarium* species produced only from 10 to 20 per cent infected plants. The reasons for the low number of isolations of *F. eumartii* are discussed in detail on p. 33 and do not need to be reconsidered here.

Table 13 presents the results of the field tests. It was obvious that the inoculated seed pieces produced a higher percentage of infected and barren plants and a lower yield of total and healthy tubers than the stem-end rot seed, and the percentage of germination was also lower. It was found, however, that the severity of the stem-end rot in the seed

[1] Soil moisture is expressed as per cent of the moisture holding capacity.
[2] Temperature records were missing from June 27 to July 3.

TABLE 13.—*Comparison of stem-end-rot seed tubers and inoculated seed tubers*

Experiment 13—1922

Seed tubers	Number of seed planted	Germination	Plants barren	Plants with infected tubers	Plants producing marketable tubers	Average weight of all tubers per plant	Weight of infected tubers	Weight of healthy marketable tubers	Yield based on controls as 100 per cent	
									Total yield	Marketable yield
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Grams*	*Per cent*	*Per cent*		
Controls	124 (680)[1]	94.4	0.0	11.1	90.5	319	2.0	84.0	100.	100.
Slight stem-end rot	135	75.5	9.8	20.5	72.0	188	11.0	61.0	59.	45.
Serious stem-end rot	135	61.4	15.4	29.7	56.0	148	20.0	52.0	46.	28.
Inoculated	270	55.5	24.6	52.6	18.0	79	64.0	17.0	24.	5.

[1]The percentage of germination of controls was based upon 680 seed pieces. All other data on controls were based upon the individual results of 124 hills.

could be correlated with the amount of disease and the resulting yield of healthy tubers. Undoubtedly much of the seed contained organisms not isolated in the cultural studies. Examination of the plants in the field showed that the disease resulted in most cases from rotting the seed piece and the advance of the disease up the stem. This does not necessarily mean, however, that the causal organism was always present in the seed. The rotting of the seed due to the action of any one of a number of organisms might permit the entrance of *Fusarium eumartii* from the soil.

Relation of Stem-End-Rot Seed to the Disease

Experiment 14, 1923

Further experiments with stem-end rot seed were conducted in 1923 at various points in western Nebraska to determine if possible the relation of weather conditions to the disease, and the transmission of the disease from stem-end rot tubers.

Methods:—The locations of the experimental plots and the dates of planting were as follows:

Plot A—Planted May 16, Scotts Bluff substation, Mitchell, Nebraska, dry land culture.

Plot B—Planted May 16, Scotts Bluff substation, Mitchell, Nebraska, irrigated.

Plot C—Planted June 8, Scotts Bluff substation, Mitchell, Nebraska, irrigated.

Plot D—Planted June 4, August Gadeken's farm, Bushnell, Nebraska.

All the plots were dug September 15 to 20.

The seed was from a bin selection and contained all types of stem-end rot. Each tuber was cut lengthwise into 4 seed pieces for planting in the 4 plots. Notes were taken on the type of injury and the tubers were divided into 7 different groups based on the type of infection. Notes were made at various times thruout the season and records taken of air and soil temperature and soil moisture.

Results:—The detailed results of this experiment are shown in Table 14. The differences in the various plots are not so evident or consistent as they were in Experiment 12. It must be remembered, however, that the bin-selected stem-end rot seed tubers provided a more variable factor as a basis for the experiment than the inoculated tubers in Experiment 12.

In addition the environmental conditions thruout the season were rather abnormal. The temperature conditions for Plots A, B, and C were practically the same except that Plot C was planted about 3 weeks later. The mean monthly temperatures for these 3 plots were June 66.7°, July 74.7°, and August 69.5° F. During many weeks of the experiment the temperature was at the optimum for the development of the disease in these 3 plots. The temperature in Plot D was below the optimum for the disease during most of the season, the mean monthly temperatures being—June 64.2°, July 72.6°, August 67.6°. When the mean weekly temperatures were calculated for these plots for the entire period of the experiment, it was found that the temperature of Plot D was consistently 2° to 4° below that of Plots A, B, and C. The rainfall for the months of May, June, July, and August was considerably above normal for all plots. Plot D had a total of 14.11 inches precipitation, which was 59 per cent above normal, while Plots A, B, and C had 13.64 inches, or 39 per cent above normal. As a result of the high rainfall and the differences of the moisture holding capacities of the soil of the various plots, it was found that the moisture content based on the moisture equivalent was less in the irrigated plots during most of the experiment than in the plots with dry land culture.

The results presented in Table 14 show that the disease was not as severe in Plot D, with temperatures slightly below the optimum for the disease, as in the other plots. The low germination in Plot D, however, was due chiefly to poor cultural conditions, as indicated by the controls. Plot C, planted two weeks later than Plots A and B, had a smaller number of plants with infected tubers and a smaller percentage of tubers showing stem-end rot, along with a lower total yield. This was probably due to the fact that this plot was dug at the same time as Plots A and B, and therefore the duration of the experiment was two weeks shorter. These results correspond with many observations made in commercial fields, that is, that early digging of infected fields will often decrease the percentage of stem-end rot tubers.

No correlation could be made in this experiment between the type of infection occurring in the seed and the resulting disease. Therefore, the detailed results are not presented.

No results are presented on the percentage of plants wilting in the field, because a rather severe attack of early blight during August complicated the symptoms and the detailed

TABLE 14.—*Results of field experiments with stem-end-rot seed*

Experiment 14—1923

Plot	Seed	Number of seed planted	Germination	Plants barren	Plants with infected tubers	Plants producing marketable tubers	Average weight of tubers per plant	Weight of infected tubers	Weight of healthy tubers	Yield based on controls as 100 per cent	
										Total yield	Marketable yield
			Per cent	*Per cent*	*Per cent*	*Per cent*	*Grams*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Plot A	Control	50	95.0	0	4.0	98.0	362.	0.7	80.1		
	Stem-end rot	218	86.2	4.7	79.2	65.9	427.	44.0	42.6	118.0	62.8
Plot B	Control	50	98.5	0	46.0	94.0	454.	8.8	63.0		
	Stem-end rot	248	94.7	2.9	80.0	51.0	326.	47.2	35.2	71.8	40.1
Plot C	Control	50	94.0	0	14.0	90.0	410	2.6	75.8		
	Stem-end rot	248	81.8	6.8	58.6	61.0	290	27.9	50.4	70.7	46.9
Plot D	Control	150	71.3	0	3.5[1]	68.4[1]	186.	1.7	64.0		
	Stem-end rot	248	63.7	1.2	50.6	60.7	242.	28.0	46.3	130.2	94.3
Average	Control	300[2]	87.0	0	16.7	86.9	353.	4.3	70.9		
	Stem-end rot	962	78.4	4.0	68.4	59.1	321.	38.8	42.8	90.7	55.2

[1]Based on 57 hills.
[2]Total seed planted.

results are therefore omitted. Slight wilting was rather general, however, in all plots. There were only a few plants that wilted completely, probably due to the high soil moisture content occurring thruout the period of the experiment.

The average for all 4 plots shows the general effect of the disease to be rather severe. The yield of marketable tubers was much less in every plot from the infected seed than from the healthy seed. While the total yield was sometimes greater with the infected seed, it must be remembered that the yields shown in Table 14 are based on the number of plants. If the data were based on the number of seed planted, and the effect of missing hills on the yield of surrounding plants taken into consideration, it would be found that both the total yield and the yield of marketable tubers would be greatly reduced for the stem-end rot seed.

Relation of Infested Soil and Stem-End-Rot Seed to the Disease

Experiment 15, 1923

Previous experiments have shown that the planting of stem-end rot seed resulted in an increase in the disease and a corresponding decrease in yield. The following experiment was planned to determine the effect of soil infestation as well as seed infection on the amount of disease.

Methods:—Two plots were selected on the same farm at Hemingford, Nebraska. One of these plots had not been planted with potatoes for several years while the other had grown a crop of potatoes the previous year which had a high percentage of wilt and stem-end rot. Both stem-end rot and healthy seed were from the same sources as those used in Experiment 14. The stem-end rot seed was divided into groups according to the various types of infection, and corresponding halves of each seed piece planted in each plot. No temperature records were taken at these plots, but the mean temperature was about optimum for the disease or slightly below. The precipitation was above normal thruout the period of the experiment.

Results:—The results of this experiment are presented in Table 15. Comparing the results from infected seed and healthy seed, in both plots, the disease resulted in decreased germination, an increase in barrenness and in the number of infected plants, and an increase in the number of infected tubers, with a resulting decrease in both total and marketable yield. In comparing the two plots it was evident that infested

TABLE 15.—*Relation of infested soil and stem-end-rot seed to the disease*

Experiment 15—1923

Plot	Seed	Number of seed planted	Germination	Plants barren	Plants with infected tubers	Plants producing marketable tubers	Average weight of tubers per plant	Weight of infected tubers	Weight of healthy tubers	Yield based on controls as 100 per cent	
										Total yield	Marketable yield
			Per cent	*Per cent*	*Per cent*	*Per cent*	*Grams*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Non-infested soil[1]	Controls	123	96.0	0	17.0	100.0[2]	793.3	2.6	89.2	100.0	100.0
	Stem-end rot	403	70.0	8.1	40.3	85.8	599.0	15.5	75.9	75.5	64.3
Infested soil	Controls	112	90.0	0	7.7	100.0[2]	434.2	4.4	88.0	100.0	100.0
	Stem-end rot	403	70.0	4.1	42.0	80.0	323.0	20.8	71.6	74.5	60.6

[1]Non-infested soil refers to that not known to have been planted to potatoes for 3 years; it was probably slightly infested.
[2]Based on individual hill counts of 53 control plants (every alternate 5 plants in the row).

soil caused a considerable increase in disease with a resulting decrease in yields. The healthy seed planted on infested soil showed only a slight increase in the number of infected tubers over the healthy seed on non-infested soil, and the number of infected plants was even less; however, the yield both total and marketable was only about half as great. The total yield of the stem-end rot seed was considerably higher on the non-infested soil and the amount of infection was correspondingly lower. The total yield of the controls on the infested soil was only 74 per cent of that on non-infested soil, while the stem-end rot seed yielded only 54 per cent as much on infested soil. The decrease in yields on the infested soil was partially due to the preparation of the seed bed. In this plot the soil had been planted to oats and was plowed just before planting. The other had been prepared for potatoes earlier in the spring. The results shown in Table 15 are based on germinated seed, but yields based on the number of seed planted would show even greater differences. Because of the high soil moisture content thruout the experiment, complete wilting occurred in only a small number of plants. Slight wilting was very prevalent in plants from the infected seed in both plots. As in previous experiments, the type of infection in the seed piece could not be correlated with the resulting disease.

Discussion of Field Experiments

Different methods of seed inoculations were tested and it was found that immersion of freshly cut seed in a spore suspension of the pathogene resulted in the greatest amount of disease. Allowing such cut seed to callus over for several days before inoculating did not prevent infection, however. Inoculations with 0.5 c. c. of spore suspension in a wound did not reduce germination greatly but did cause considerable disease.

Tests made under different environmental conditions showed that the results of such inoculation experiments could be correlated with the results obtained in the greenhouse under control conditions. High soil temperatures early in the season resulted in a great decrease in germination. A high percentage of wilting occurred when the air and soil temperatures were optimum for the disease and such wilting could be correlated with low soil moisture contents in midseason. The amount of stem-end rot occurring in the tubers could not be correlated with weather conditions. Mean tem-

peratures below the optimum for the disease decreased the amount of disease.

Experiments with stem-end rot seed showed that such seed could transmit the disease. Stem-end rot seed on non-infested soil yielded about the same as healthy seed on infested soil.

Both stem-end rot seed and infested soil caused the same type of disease as that produced by inoculating the seed, altho the amount of disease was not as great. No correlation could be made between the type of infection in the stem-end rot seed and the resulting disease, nor could either of these be correlated with the organisms isolated from such seed tubers.

The disease resulted in decreased germination, increased barrenness, a large number of plants producing infected tubers, and a decreased total yield, with a high percentage of stem-end rot tubers.

With a high soil moisture content the wilt symptoms were not very evident, but the disease under these conditions often resulted in a large number of stem-end rot tubers such as occurred in the greenhouse under controlled conditions. Early digging tended to decrease the amount of stem-end rot in the tubers.

It was evident from these experiments that the disease can be transmitted by either infested soil or seed. Even when the seed tubers did not yield *Fusarium eumartii* in culture, it was evident that such seed produced a weaker plant which was apparently more susceptible to infection from the soil. The only control measure which recommends itself is the use of disease-free seed on non-infested soil, with early digging of affected plants.

RELATION OF TEMPERATURE AND RELATIVE HUMIDITY TO TUBER ROT IN STORAGE

Preliminary Tests

Healthy Bliss Triumph tubers were inoculated by introducing the inoculum into a small wound, and others were inoculated by dipping unwounded tubers in a spore suspension of the pathogene. These tubers were placed in desiccators containing sulphuric acid in concentrations necessary to obtain a range from 20 to 100 per cent in the relative humidity. The tops were sealed and the desiccators were placed at a temperature of 25° C.

It was found that infection occurred only when the tubers had been wounded. The lack of aeration in the containers resulted in considerable black heart, and apparently inhibited the progress of the pathogene so that no differences could be noted at the various relative humidities employed.

Further tests were made to determine whether or not *Fusarium eumartii* was capable of penetrating the unwounded epidermis of the potato tuber. These tests were made by treating healthy tubers with a solution of mercuric chloride, washing them in sterile water, and placing them in moist chambers at 25° C. after making the following treatments:

1. Tubers wounded with sterile scalpel by making a cut ¼ inch deep.
   (a) Tubers immersed in a spore suspension of *F. eumartii.*
   (b) One sq. cm. of an agar culture of *F. eumartii* was inverted over the wound, with the mycelium in contact with the tuber.
2. Tubers unwounded.
   (a) Same as above.
   (b) Same as above.

Infection occurred with both methods of inoculation in wounded tubers, but no infection was ever obtained in unwounded tubers.

Tuber Inoculations

Experiment 16, March 28—May 11, 1923

In this experiment an attempt was made to study the pathogenicity of *Fusarium eumartii* on tubers at various temperatures and relative humidities, and also to allow for sufficient aeration to eliminate black heart and to produce more normal conditions than were present in the preliminary tests.

Methods:—The experiment was conducted at 5 different temperatures: 5°, 10°, 15°, 20°, and 25° C., with 4 relative humidities at each temperature.

The principle employed in the preliminary tests of using solutions of sulphuric acid in varying concentrations to give the desired relative humidity was again used. Large battery jars were employed containing 750 c. c. of sulphuric acid at the necessary specific gravity to give 10.5, 38.0, 70.4 and 100.0 per cent of relative humidity. To allow for aeration the jars instead of being sealed were closed with a thick cotton pack covered with cheesecloth. This provided for a sterile atmosphere without asphyxiating the tubers. It was expected that the specific gravity of the solutions would change

considerably during the experiment and thus change the relative humidity but by using only 4 degrees of saturation deficit it was hoped that this change would not result in overlapping. At the end of the experiment the concentration of the sulphuric acid was determined with Twaddell hygrometers, and the specific gravity and saturation deficit calculated. The following changes were found to have taken place.

PER CENT RELATIVE HUMIDITY

Initial at all temperatures	Final				
	5°	10°	15°	20°	25°
Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
70.4	69.8	68.0	64.9	62.7	62.7
38.0	42.5	42.5	44.4	41.0	49.0
10.5	14.0	19.0	20.0	22.4	23.9

Altho the above data show that the change in the relative humidity in each set at different temperatures was considerable, the extremes did not overlap and the same relative position of the series remained. The set at 100 per cent relative humidity remained constant.

All tubers used were treated with $HgCl_2$ and washed in sterile water. Four tubers were placed in each jar. Two tubers were inoculated by making a small hole with a cork borer, 3x5 mm. in the side of the tuber and placing in this cavity 2 drops of a spore suspension of *Fusarium eumartii* from a 10-day old culture on hard oat agar. The other two tubers were inoculated by inserting spores and mycelium into a small wound made with a sterile scalpel at the stem end of the tuber. Immediately after inoculating, all tubers were put in the jars, which were then placed at the various temperatures.

Results:—The results of this experiment are presented in Table 16. The amount of rotting was about the same with both methods of inoculation. The greatest amount of rot occurred at 25° C. with 100 per cent relative humidity (Plate 2). At 70 per cent relative humidity the extent of rot was about the same as at 100 per cent. There was a decided decrease, however, at the lower humidities. At 10° rotting occurred at the 3 highest relative humidities while no infection took place at 10 per cent. No infection occurred at 5° at any humidity, and the results at this temperature are therefore not recorded in Table 16; the fungus developed in the wounded tissue but seemed unable to penetrate the healthy tissue.

TABLE 16.—*Relation of temperature and relative humidity to the rotting of tubers in storage*

Experiment 16

Extent of rotting expressed in mm. diameter of rot (surface x depth)

Method of inoculation	25° C.				20° C.				15° C.				10° C.				
	100%	70%	40%	10%	100%	70%	40%	10%	100%	70%	40%	10%	100%	70%	40%	10%	
By wound at stem-end	40x20	40x25	Complete	30x30	20x12	15x10	15x10	20x20	13x7	15x10	5x4	4x4	12x8	10x12	15x4		
	40x30	40x35	30x30	30x30	20x15	15x12	15x10	15x10	13x7	13x 7	10x4	7x10	12x8	10x8	10x5		
By placing 2 drops of a spore suspension in cavity	Complete	47x45	27x25	27x25	22x12	22x10	17x12	15x5	12x10	12x5	0	1x1	10x2	7x5	7x1		
	Complete	42x35	Complete	37x15	17x15	27x15	15x5	Contaminated	10x10	Contaminated	0	0	10x2	3x7	7x1		No infection

All tubers in the experiment except those completely rotted showed healthy eyes and in most instances the sprouts were well started. No fungus growth occurred on the surface of the potatoes except at complete saturation. In most cases a large cavity was formed, and this cavity was surrounded by an interwoven mass of fungus hyphae and starch grains.

In general, an increase of 5° C. in temperature resulted in as great an increase in rotting as an increase from 10 per cent relative humidity to saturation at any specified temperature. We may conclude from this experiment that the control of this rot can be obtained by submitting tubers to a temperature of 5°, or even slightly higher temperatures provided the relative humidity is low.

Stem-End-Rot Tubers

Experiment 17, January 21—March 21, 1923

In this experiment, tubers showing various types of stem-end rot obtained from wilted hills in the field[1] were submitted to different temperatures and relative humidities to determine if possible the effect of these factors on the advance of the disease.

Methods:—The temperatures used were 5°, 10°, 15°, 20°, and 25° C. Three relative humidities, 20, 60, and 100 per cent, were obtained at each temperature as in the previous experiment. At the end of the experiment the solutions were again tested and the low relative humidity set was found to vary from 20 to 30 per cent, the 60 per cent varied from 50 to 60 per cent, and the 100 per cent remained constant.

Each tuber used was carefully examined for external evidences of stem-end rot, and notes taken. Twenty-five whole tubers were submitted to various temperatures and relative humidities. Seventeen other tubers were halved and notes taken on the type of internal injury. The cut surfaces were then dipped in paraffin, and the corresponding halves placed at different temperatures and relative humidities.

Results:—Healthy tubers treated in a similar way to the infected tubers remained healthy thruout the experiment, even at 25° C. with 100 per cent relative humidity.

No clear-cut comparison could be made at different temperatures and relative humidities regarding the increase in stem-end rot of the whole tubers. Only 3 of the 25 tubers

[1]The author is indebted to the Horticultural Department for the selection of these infected tubers.

showed any increase in the external appearance of the disease. It is possible that the disease advanced internally and could not be detected on the outside.

Eleven of the 17 halved tubers showed a distinct increase of infected tissue. Four of the 11 halved tubers placed at various humidities at 5° and 10° showed no advance in rotting while the other halves of these 4 tubers when placed at corresponding relative humidities at 25° C. showed a distinct advance, and in 2 of these complete rotting occurred. At 15° a slight advance in rotting occurred in 2 of the 3 halves used, while the other halves at corresponding relative humidities at 25° showed a much greater increase; in one instance complete rotting occurred. At 20°, one halved tuber showed a slight advance of rot at 100 per cent humidity and one at 20 per cent showed no increase. The other halves placed at corresponding humidities at 25° underwent complete rot. Two halves placed at 20 per cent saturation at 20° and 25° showed a slight advance, while the other halves at 100 per cent saturation rotted completely at these temperatures.

It was evident from this experiment that stem-end rot and internal discoloration produced in the field may increase in extent when placed in storage with conditions favorable for the disease. A temperature of 10° C. or below was sufficient to hold the disease in check under all conditions of relative humidity. In the previous experiment with inoculated tubers this temperature prevented the advance of the disease only with a low relative humidity. Temperatures higher than 10° tend to increase the extent of rotting in infected tubers in proportion to the increase in temperature, up to 25°. Temperature is more important than relative humidity altho as the humidity approaches saturation more favorable conditions for the disease are afforded.

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PLATE 1

Tubers showing various types of stem-end rot and vascular discoloration, All these tubers were produced on wilted plants which had been inoculated with pure cultures of *Fusarium eumartii.*

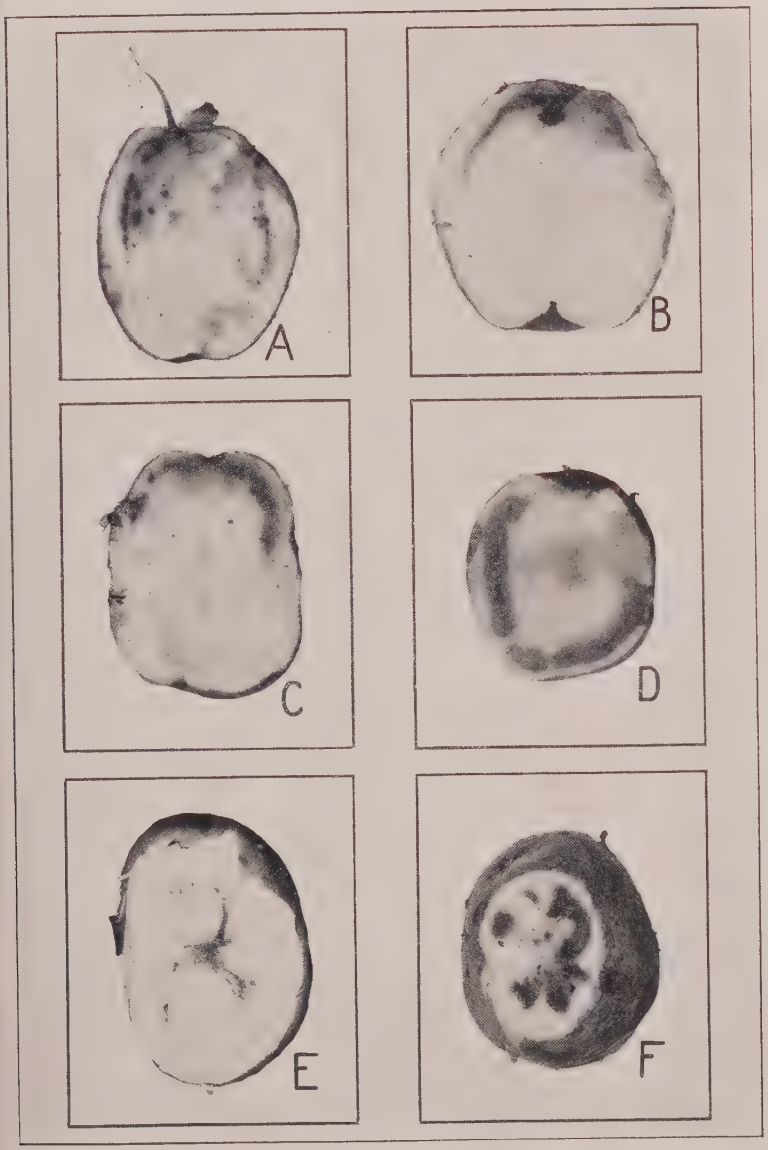

A
B
C
D
E
F

PLATE 2

Tubers inoculated with *Fusarium eumartii* and placed under the following conditions of temperature and relative humidity, for 6 weeks. Experiment 16, p. 78.

A.—25° C.—10 per cent relative humidity.

B.—20° C.—10 per cent relative humidity.

C.—15° C.—10 per cent relative humidity.

D.—25° C.—100 per cent relative humidity.

E.—20° C.—100 per cent relative humidity.

F.—15° C.—100 per cent relative humidity.

G.—10° C.—100 per cent relative humidity.

Other tubers in Experiment 16 showed degrees of injury midway between the extremes shown here.

Tubers A and B are typical of stem-end rot tubers as they sometimes occur in the field, without any vascular discoloration.

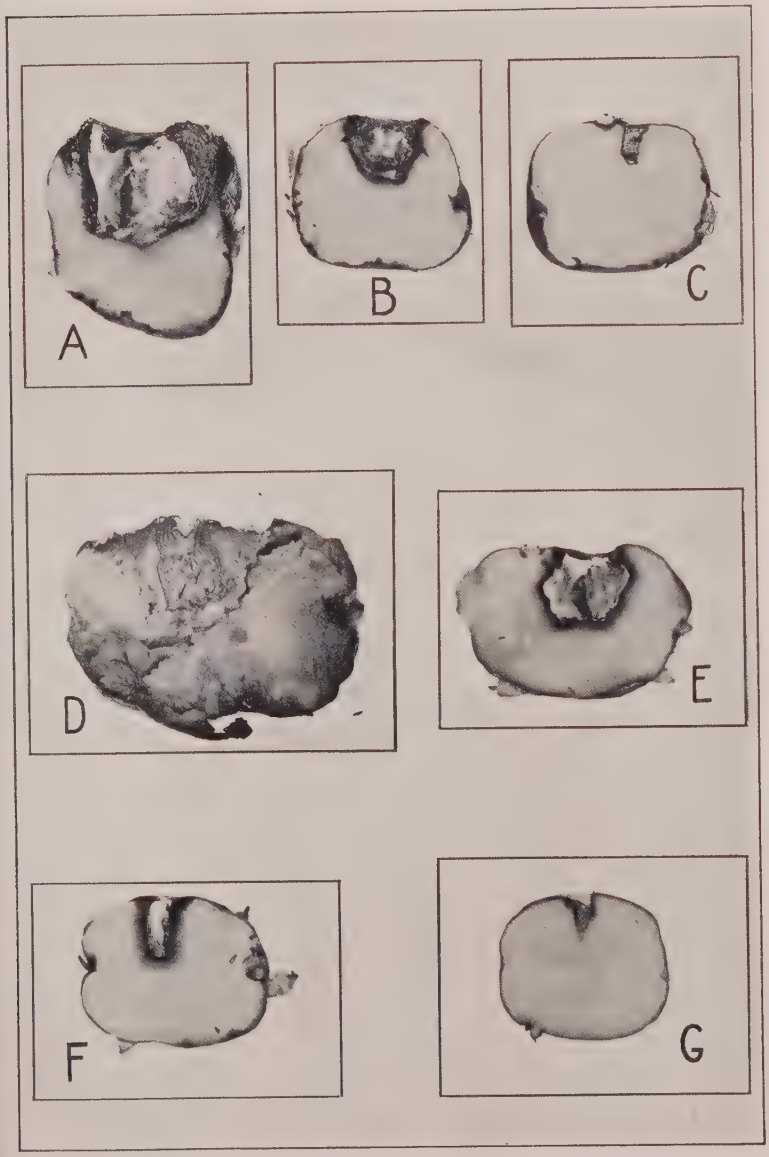

A
B
C
D
E
F
G

PLATE 3

A and B.—Tubers inoculated at the stem end with *Fusarium eumartii*. This type of rot is typical of that often occurring in the field except for the external growth of mycelium.

C.—Representative plants from Experiment 3 showing the effect of soil temperature on the disease produced by inoculated soil. One control plant grown at 20° C. is shown at the left.

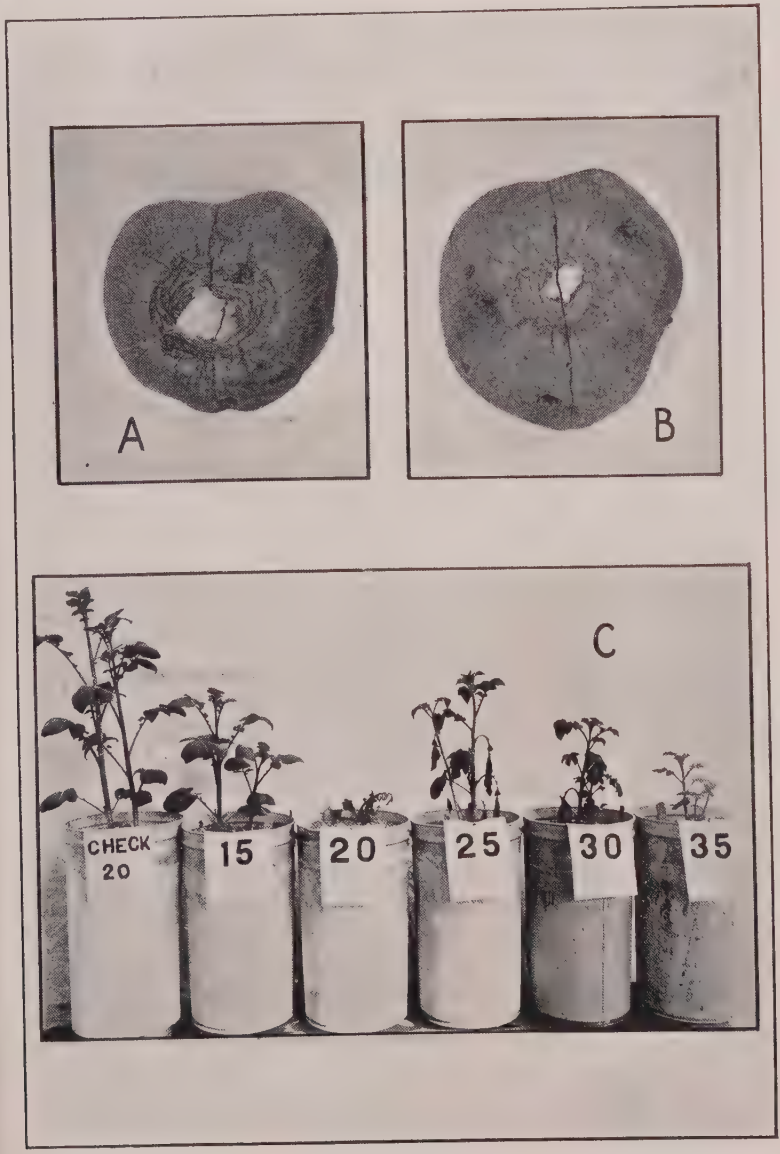

A
B
C
CHECK
20
15
20
25
30
35

PLATE 4

Results from soil moisture experiments with soil inoculation. Experiment 6.

A—1—Control, and A—2—Inoculated, at 1 Moisture equivalent.

B—1—Control, and B—2—Inoculated, at 1¼ Moisture equivalent.

C—1—Control, and C—2—Inoculated, at 1½ Moisture equivalent.

A-1
A-2
B-1
B-2
C-1
C-2

PLATE 5

A.—Showing root infection with inoculated soil, (page 59). Photograph taken thru the glass wall of the container in which plant was growing in sand inoculated with *Fusarium eumartii.*

(X) shows infected root tips, and (H) healthy portion of root farther back from the infected tips.

B.—Culture 3 days old made on potato agar from extreme edge of discolored vascular tissue in an infected tuber. The white circle indicates the size of the piece of tissue used. The culture was sterile and the dark discoloration of the media surrounding the tissue is typical of isolations from such tissue.

A
B

PLATE 6

A.—Plant infected by the inoculation of 2 roots as described on p. 61.

B.—Showing the large healthy root system. (X) indicates the inoculated roots from which the disease progressed into the stem and downwards to the healthy seed piece producing a vascular discoloration in the seed piece.

C.—Section of the upper stem of the same plant.

A
B
C

PLATE 7

A.—Healthy control plant at the left, with 2 infected plants completely wilted at the right, from Soil Inoculation Experiment 7. The roots of the infected plants are almost entirely destroyed, the seed pieces have started to decay, but the vascular discoloration in the seed pieces may have been produced in the same way as that shown in Plate 6.

B.—Infected and control plant from Seed Inoculation Experiment 8. Note the complete decay at the base of the stem as contrasted with the type of infection shown in A from soil inoculation.

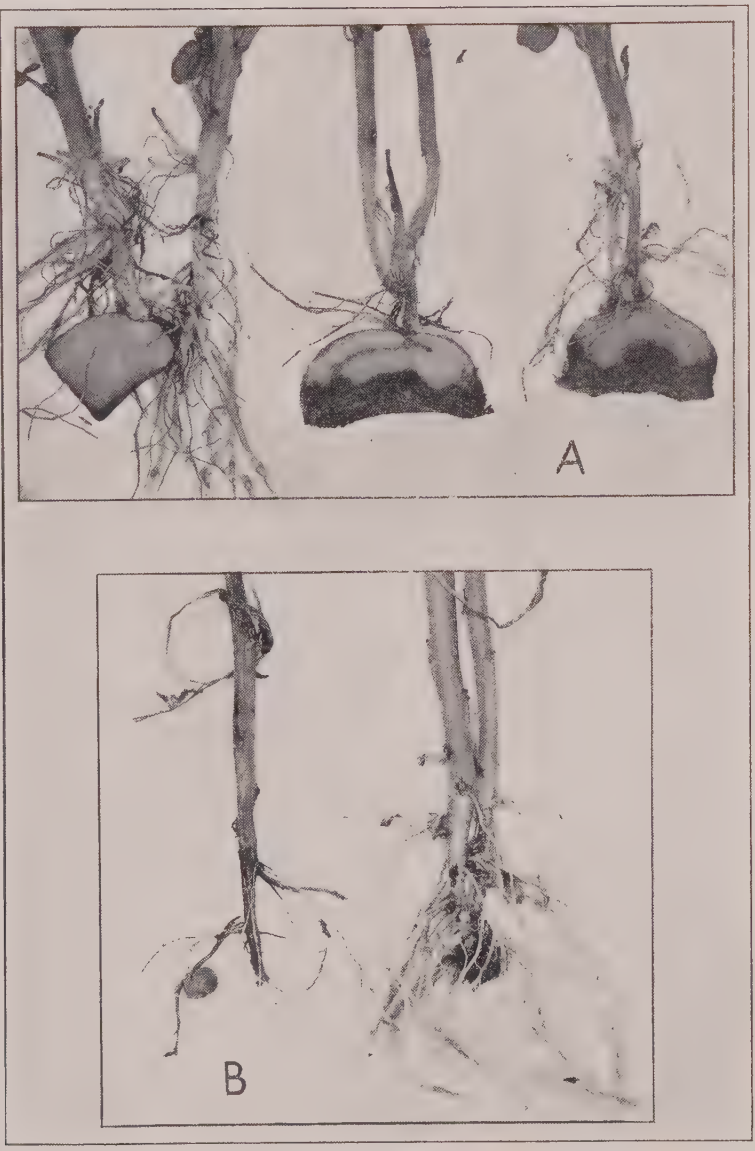

A
B

PLATE 8

A.—Tangential section thru a small root showing the outer cells containing the organism. x 240.

B.—Transverse section of infected root showing the disintegration of the cortex in advance of the organism which could not be detected in this section. x 66.

C.—Tangential section of stem at base of an infected root. The vascular cylinder of the root is indicated at the extreme right. The organism was abundant in the cortex at this point. x 165.

D.—Tangential section of potato tuber showing necrosis of the cells and the presence of the deep staining granular substance. A strand of vascular tissue is indicated at the extreme right. x 145.

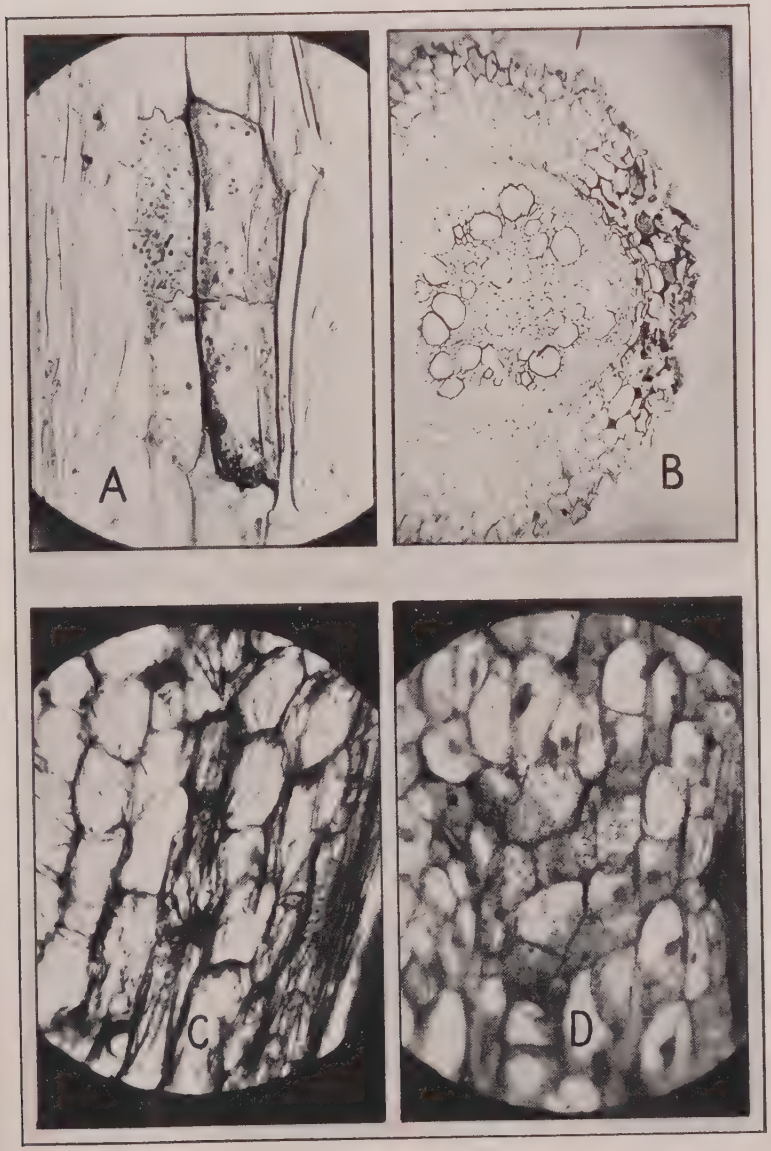

A
B
C
D

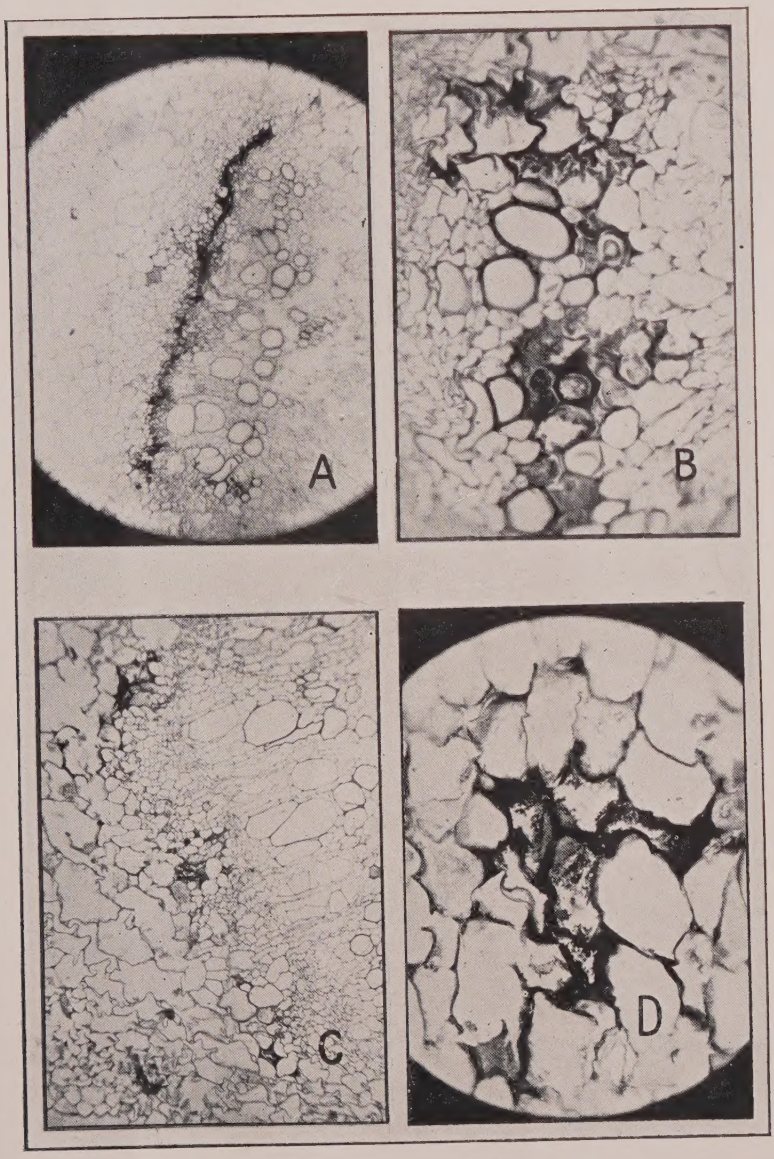
PLATE 9

A.—Transverse section thru a vascular bundle of the stem 2 inches above point shown in Plate 8, C. The organism could not be detected at this point. Necrosis of the cambium and phloem is apparent, however, in advance of the organism. x 50.

B.—Another bundle of the section shown in A with necrosis of the xylem tissue, x 185.

C.—The third bundle of the section shown in A and B, with necrosis of the external phloem. x 72.

D.—Tangential section thru the pith 6 inches above point shown in A, B, and C. The disintegration of the pith cells in advance of the organism is shown with the thickening of the cell walls and the presence of the granular substance which stains deeply as does the cell walls. x 145.


A
B
C
D

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